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- (74) Agents: **ANTLER, Adriane, M. et al.**; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).
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- (71) Applicant: **UNIVERSITY OF CONNECTICUT HEALTH CENTER [US/US]**; 263 Farmington Avenue, Farmington, CT 06030 (US).
- (72) Inventor: **SRIVASTAVA, Pramod, K.**; 70 Pheasant Run, Avon, CT 06001 (US).
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(54) Title: **COMPLEXES OF ALPHA (2) MACROGLOBULIN AND ANTIGENIC MOLECULES FOR IMMUNOTHERAPY**

(57) Abstract: The present invention relates to complexes of alpha (2) macroglobulin associated with antigenic molecules for use in immunotherapy. The invention relates to methods for using such compositions in the diagnosis and treatment of immune disorders, proliferative disorders, and infectious diseases.

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COMPLEXES OF ALPHA (2) MACROGLOBULIN AND ANTIGENIC MOLECULES FOR IMMUNOTHERAPY

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invention.

1. INTRODUCTION

The present invention relates to complexes of alpha (2) macroglobulin associated
10 with antigenic molecules for use in immunotherapy. The invention relates to methods for
using such compositions in the diagnosis and treatment of immune disorders, proliferative
disorders, and infectious diseases.

2. BACKGROUND OF THE INVENTION

2.1. HEAT SHOCK PROTEINS

Heat shock proteins (HSPs), also referred to as stress proteins, were first identified
as proteins synthesized by cells in response to heat shock. Hsps have classified into five
families, based on molecular weight, Hsp100, Hsp90, Hsp70, Hsp60, and smHsp. Many
20 members of these families were found subsequently to be induced in response to other
stressful stimuli including nutrient deprivation, metabolic disruption, oxygen radicals, and
infection with intracellular pathogens (see Welch, May 1993, Scientific American 56-64;
Young, 1990, Annu. Rev. Immunol. 8:401-420; Craig, 1993, Science 260:1902-1903;
Gething *et al.*, 1992, Nature 355:33-45; and Lindquist *et al.*, 1988, Annu. Rev. Genetics
25 22:631-677).

Heat shock proteins are among the most highly conserved proteins in existence. For
example, DnaK, the Hsp70 from *E. coli* has about 50% amino acid sequence identity with
Hsp70 proteins from excoiates (Bardwell *et al.*, 1984, Proc. Natl. Acad. Sci. 81:848-852).
The Hsp60 and Hsp90 families also show similarly high levels of intra-family conservation
30 (Hickey *et al.*, 1989, Mol. Cell. Biol. 9:2615-2626; Jindal, 1989, Mol. Cell. Biol. 9:2279-
2283). In addition, it has been discovered that the Hsp60, Hsp70 and Hsp90 families are
composed of proteins that are related to the stress proteins in sequence, for example, having
greater than 35% amino acid identity, but whose expression levels are not altered by stress.

Studies on the cellular response to heat shock and other physiological stresses
35 revealed that the HSPs are involved not only in cellular protection against these adverse
conditions, but also in essential biochemical and immunological processes in unstressed

cells. Hsps accomplish different kinds of chaperoning functions. For example, members of the Hsp70 family, located in the cell cytoplasm, nucleus, mitochondria, or endoplasmic reticulum (Lindquist *et al.*, 1988, Ann. Rev. Genetics 22:631-677), are involved in the presentation of antigens to the cells of the immune system, and are also involved in the transfer, folding and assembly of proteins in normal cells. Hsps are capable of binding proteins or peptides, and releasing the bound proteins or peptides in the presence of adenosine triphosphate (ATP) or low pH.

2.2. IMMUNOGENICITY OF HSP-PEPTIDE COMPLEXES

Srivastava *et al.* demonstrated immune response to methylcholanthrene-induced sarcomas of inbred mice (1988, Immunol. Today 9:78-83). In these studies, it was found that the molecules responsible for the individually distinct immunogenicity of these tumors were glycoproteins of 96kDa (gp96) and intracellular proteins of 84 to 86kDa (Srivastava *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:3407-3411; Ullrich *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:3121-3125). Immunization of mice with gp96 or p84/86 isolated from a particular tumor rendered the mice immune to that particular tumor, but not to antigenically distinct tumors. Isolation and characterization of genes encoding gp96 and p84/86 revealed significant homology between them, and showed that gp96 and p84/86 were, respectively, the endoplasmic reticular and cytosolic counterparts of the same heat shock proteins (Srivastava *et al.*, 1988, Immunogenetics 28:205-207; Srivastava *et al.*, 1991, Curr. Top. Microbiol. Immunol. 167:109-123). Further, Hsp70 was shown to elicit immunity to the tumor from which it was isolated but not to antigenically distinct tumors. However, Hsp70 depleted of peptides was found to lose its immunogenic activity (Udono and Srivastava, 1993, J. Exp. Med. 178:1391-1396). These observations suggested that the heat shock proteins are not immunogenic per se, but form noncovalent complexes with antigenic peptides, and the complexes can elicit specific immunity to the antigenic peptides (Srivastava, 1993, Adv. Cancer Res. 62:153-177; Udono *et al.*, 1994, J. Immunol., 152:5398-5403; Suto *et al.*, 1995, Science 269:1585-1588).

Noncovalent complexes of HSPs and peptide, purified from cancer cells, can be used for the treatment and prevention of cancer and have been described in PCT publications WO 96/10411, dated April 11, 1996, and WO 97/10001, dated March 20, 1997 (U.S. Patent No. 5,750,119 issued April 12, 1998, and U.S. Patent No. 5,837,251 issued November 17, 1998, respectively, each of which is incorporated by reference herein in its entirety). The isolation and purification of stress protein-antigen complexes has been described, for example, from pathogen-infected cells, and used for the treatment and prevention of infection caused by the pathogen, such as viruses, and other intracellular pathogens, including bacteria, protozoa, fungi and parasites (see, for example, PCT Publication WO 95/24923, dated September 21,

1995). Immunogenic stress protein-antigen complexes can also be prepared by in vitro complexing of stress protein and antigenic peptides, and the uses of such complexes for the treatment and prevention of cancer and infectious diseases has been described in PCT publication WO 97/10000, dated March 20, 1997 (U.S. Patent No. 6,030,618 issued February 29, 2000. The use of stress protein-antigen complexes for sensitizing antigen presenting cells in vitro for use in adoptive immunotherapy is described in PCT publication WO 97/10002, dated March 20, 1997 (see also U.S. Patent No. 5,985,270 issued November 16, 1999).

2.3. ALPHA (2) MACROGLOBULIN RECEPTOR

The α -macroglobulins are members of a protein superfamily of structurally related proteins which also comprises complement components C3, C4 and C5. The human plasma protein alpha (2) macroglobulin (α 2M) is a 720 kDa homotetrameric protein primarily known as proteinase inhibitor and plasma and inflammatory fluid proteinase scavenger molecule (for review see Chu and Pizzo, 1994, Lab. Invest. 71:792). Alpha (2) macroglobulin is synthesized as a 1474 amino acid precursor, the first 23 of which function as a signal sequence that is cleaved to yield a 1451 amino acid mature protein (Kan *et al.*, 1985, Proc. Natl. Acad. Sci. U.S.A. 82:2282-2286).

Alpha (2) macroglobulin promiscuously binds to proteins and peptides with nucleophilic amino acid side chains in a covalent manner (Chu *et al.*, 1994, Ann. N.Y. Acad. Sci. 737:291-307) and targets them to cells which express the α 2M receptor (α 2MR) (Chu and Pizzo, 1993, J. Immunol. 150:48). Binding of α 2M to the α 2M receptor is mediated by the C-terminal portion of α 2M (Holtet *et al.*, 1994, FEBS Lett. 344:242-246) and key residues have been identified (Nielsen *et al.*, 1996, J. Biol. Chem. 271:12909-12912).

Generally known for inhibiting protease activity, α 2M binds to a variety of proteases thorough multiple binding sites (see, e.g., Hall *et al.*, 1981, Biochem. Biophys. Res. Commun. 100(1):8-16). Protease interaction with α 2M results in a complex structural rearrangement called transformation, which is the result of a cleavage within the "bait" region of α 2M after the proteinase becomes "trapped" by thioesters. The conformational change exposes residues required for receptor binding, allowing the α 2M-proteinase complex to bind to the α 2MR. Methylamine can induce similar conformational changes and cleavage as that induced by proteinases. The uncleaved form of α 2M, which is not recognized by the receptor, is often referred to as the "slow" form (s- α 2M). The cleaved form is referred to as the "fast" form (f- α 2M) (reviewed by Chu *et al.*, 1994, Ann. N.Y. Acad. Sci. 737:291-307).

Studies have shown that in addition to its proteinase-inhibitory functions, α 2M, when complexed to antigens, can enhance the antigens' ability to be taken up by antigen presenting cells such as macrophages and presented to T cell hybridomas *in vitro* by up to two orders of

magnitude (Chu and Pizzo, 1994, Lab. Invest. 71:792), and induce T cell proliferation (Osada et al., 1987, Biochem. Biophys. Res. Commun. 146:26-31). Further evidence suggests that complexing antigen with $\alpha 2M$ enhances antibody production by crude spleen cells *in vitro* (Osada et al., 1988, Biochem. Biophys. Res. Commun. 150:883) elicits an *in vivo* antibody responses in experimental rabbits (Chu et al., 1994, J. Immunol. 152:1538-1545) and mice (Mitsuda et al., 1993, Biochem. Biophys. Res. Commun. 101:1326-1331). However, none of these studies have shown whether $\alpha 2M$ -antigen complexes are capable of eliciting cytotoxic T cell responses *in vivo*.

2.4. IMMUNOGENICITY OF HEAT SHOCK/STRESS PROTEINS

Srivastava et al. demonstrated immune response to methylcholanthrene-induced sarcomas of inbred mice (1988, Immunol. Today 9:78-83). In these studies, it was found that the molecules responsible for the individually distinct immunogenicity of these tumors were identified as cell-surface glycoproteins of 96kDa (gp96) and intracellular proteins of 84 to 86kDa (Srivastava et al., 1986, Proc. Natl. Acad. Sci. USA 83:3407-3411; Ullrich, S.J. et al., 1986, Proc. Natl. Acad. Sci. USA 83:3121-3125). Immunization of mice with gp96 or p84/86 isolated from a particular tumor rendered the mice immune to that particular tumor, but not to antigenically distinct tumors. Isolation and characterization of genes encoding gp96 and p84/86 revealed significant homology between them, and showed that gp96 and p84/86 were, respectively, the endoplasmic reticular and cytosolic counterparts of the same heat shock proteins (Srivastava et al., 1988, Immunogenetics 28:205-207; Srivastava et al., 1991, Curr. Top. Microbiol. Immunol. 167:109-123). Further, Hsp70 was shown to elicit immunity to the tumor from which it was isolated but not to antigenically distinct tumors. However, Hsp70 depleted of peptides was found to lose its immunogenic activity (Udono and Srivastava, 1993, J. Exp. Med. 178:1391-1396). These observations suggested that the heat shock proteins are not immunogenic per se, but form noncovalent complexes with antigenic peptides, and the complexes can elicit specific immunity to the antigenic peptides (Srivastava, 1993, Adv. Cancer Res. 62:153-177; Udono et al., 1994, J. Immunol., 152:5398-5403; Suto et al., 1995, Science, 269:1585-1588).

The use of noncovalent complexes of stress proteins and peptides, purified from cancer cells, for the treatment and prevention of cancer, as well as the use of such complexes in combination with adoptive immunotherapy, has been described (see U.S. Patent No. 5,750,199; U.S. Patent No. 5,830,464; Patent Cooperation Treaty ("PCT") publications WO 96/10411, dated April 11, 1996; and WO 97/10001, dated March 20, 1997; each of which is incorporated by reference herein in its entirety. The purification of stress protein-peptide complexes from cell lysates has been described previously; stress protein-peptide complexes can be isolated from pathogen-infected cells and used for the treatment and prevention of

infection caused by pathogens, such as viruses and other intracellular pathogens, including bacteria, protozoa, fungi and parasites (see PCT publication WO 95/24923, dated September 21, 1995).

5 Immunogenic stress protein-peptide complexes can also be prepared by *in vitro* complexing of stress protein and antigenic peptides, and the uses of such complexes for the treatment and prevention of infectious diseases and cancer has been described in PCT publication WO 97/10000, dated March 20, 1997. The use of heat shock proteins in combination with a defined antigen for the treatment of infectious diseases and cancer have also been described in PCT publication WO 97/06821, dated February 27, 1997. The administration of expressible polynucleotides encoding eukaryotic heat shock proteins to 10 mammalian cells for stimulating an immune response, and for treatment of infectious diseases and cancer has been described in PCT publications, WO 97/06685 and WO 97/06828, both dated February 27, 1997. The use of stress protein-peptide complexes for sensitizing antigen presenting cells *in vitro* for use in adoptive immunotherapy is described in PCT publication WO 97/10002, dated March 20, 1997. 15

2.5. ANTIGEN PRESENTATION

Major histocompatibility complex (MHC) molecules present antigens on the cell surface of antigen-presenting cells. Cytotoxic T lymphocytes (CTLs) then recognize MHC 20 molecules and their associated peptides and kill the target cell. Antigens are processed by two distinct antigen processing routes depending upon whether their origin is intracellular or extracellular. Intracellular or endogenous protein antigens, *i.e.*, antigens synthesized within the antigen-presenting cell, are presented by MHC class I (MHC I) molecules to CD8+ cytotoxic T lymphocytes. On the other hand, extracellular or exogenously synthesized antigenic determinants are presented on the cell surface of "specialized" or "professional" 25 APCs (macrophages, for example) by MHC class II molecules to CD4+ T cells (see, generally, Fundamental Immunology, W.E. Paul (ed.), New York: Raven Press, 1984). This compartmental segregation of antigen processing routes is important to prevent tissue destruction that could otherwise occur during an immune response as a result of shedding of neighboring cell MHC I antigens. 30

The heat shock protein gp96 chaperones a wide array of peptides, depending upon the source from which gp96 is isolated (for review, see Srivastava *et al.*, 1998, Immunity 8: 657-665). Tumor-derived gp96 carries tumor-antigenic peptides (Ishii *et al.*, 1999, J. Immunology 162:1303-1309); gp96 preparations from virus-infected cells carry viral epitopes (Suto and Srivastava, 1995, Science 269:1585-1588; Nieland *et al.*, 1998, Proc. 35 Natl. Acad. Sci. USA 95:1800-1805), and gp96 preparations from cells transfected with model antigens such as ovalbumin or β -galactosidase are associated with the corresponding

epitopes (Arnold *et al.*, 1995, J. Exp. Med. 182:885-889; Breloer *et al.*, 1998, Eur. J. Immunol. 28:1016-1021). The association of gp96 with peptides occurs *in vivo* (Menoret and Srivastava, 1999, Biochem. Biophys. Research Commun. 262:813-818). Gp96-peptide complexes, whether isolated from cells (Tamura *et al.*, 1997, Science 278:117-120), or reconstituted *in vitro* (Blachere *et al.*, 1997, J. Exp. Med. 186:1183-1406) are excellent
5 immunogens and have been used extensively to elicit CD8+ T cell responses specific for the gp96-chaperoned antigenic peptides.

The capacity of gp96-peptide complexes to elicit an immune response is dependent upon the transfer of the peptide to MHC class I molecules of antigen-presenting cells (Suto and Srivastava, 1995, *supra*). Endogenously synthesized antigens chaperoned by gp96 in the
10 endoplasmic reticulum [ER] can prime antigen-specific CD8+ T cells (or MHC I-restricted CTLs) *in vivo*; this priming of CD8+ T cells requires macrophages. However, the process whereby exogenously introduced gp96-peptide complexes elicit the antigen-specific CD8+ T cell response is not completely understood since there is no established pathway for the
15 translocation of extracellular antigens into the class I presentation machinery. Yet antigenic peptides of extracellular origin associated with HSPs are somehow salvaged by macrophages, channeled into the endogenous pathway, and presented by MHC I molecules to be recognized by CD8+ lymphocytes (Suto and Srivastava, 1995, *supra*; Blachere *et al.*, 1997, J. Exp. Med. 186:1315-22).

Several models have been proposed to explain the delivery of extracellular peptides for antigen presentation. One proposal, known as the "direct transfer" model, suggests that HSP-chaperoned peptides are transferred to MHC I molecules on the cell surface of
20 macrophages for presentation to CD8+ T lymphocytes. Another suggestion is that soluble extracellular proteins can be trafficked to the cytosol via constitutive macropinocytosis in bone marrow-derived macrophages and dendritic cells (Norbury *et al.*, 1997, Eur. J. Immunol. 27:280-288). Yet another proposed mechanism is that HSPs are taken up by the
25 MHC class I molecules of the macrophage, which stimulate the appropriate T cells (Srivastava *et al.*, 1994, Immunogenetics 39:93-98. Others have suggested that a novel intracellular trafficking pathway may be involved for the transport of peptides from the extracellular medium into the lumen of ER (Day *et al.*, 1997, Proc. Natl. Acad. Sci. 94:8064-
30 8069; Nicchitta, 1998, Curr. Opin. in Immunol. 10:103-109). Further suggestions include the involvement of phagocytes which (a) possess an ill-defined pathway to shunt protein from the phagosome into the cytosol where it would enter the normal class I pathway; (b) digest ingested material in lysosomes and regurgitate peptides for loading on the surface to
35 class I molecules (Bevan, 1995, J. Exp. Med. 182:639-41).

Still others have proposed a receptor-mediated pathway for the delivery of extracellular peptides to the cell surface of APS for antigen presentation. In view of the

extremely small quantity of gp96-chaperoned antigenic peptides required for immunization (Blachere *et al.*, 1997, *supra*), and the strict dependence of immunogenicity of gp96-peptide complexes on functional antigen presenting cells (APCs) (Udono *et al.*, 1994, Proc. Natl. Acad. Sci. U.S.A. 91:3077-3081), APCs had been proposed to possess receptors for gp96 (Srivastava *et al.*, 1994, Immunogenetics 39:93-98). Preliminary microscopic evidence
5 consistent with such receptors has been recently obtained (Binder *et al.*, 1998, Cell Stress & Chaperones 3 (Supp.1):2.; Arnold-Schild *et al.*, 1999, J. Immunol. 162: 3757-3760; and Wassenberg *et al.*, 1999, J. Cell Sci. 1:12). One hypothesis is that the mannose receptor is used in the uptake of gp96, but no mechanism has been proposed for the non-glycosylated HSPs, such as Hsp70 (Ciupitu *et al.*, 1998, J. Exp. Med., 187:685-691).
10

The identification and characterization of specific molecules involved in HSP-mediated antigen presentation of peptides, could provide useful reagents and techniques for eliciting specific immunity by HSP and HSP-peptide complexes, and for developing novel diagnostic and therapeutic methods.

15 Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

3. SUMMARY OF THE INVENTION

20 The present invention provides complexes comprising alpha (2) macroglobulin ("α2M") and methods for their use in immunotherapy. The invention is based, in part, on the Applicant's discovery that α2M directly competes for the binding of heat shock protein gp96 to the α2M receptor, indicating that α2M and HSPs may bind to a common recognition site on the alpha (2) macroglobulin receptor. Thus, because HSPs and α2M have a number of
25 common functional attributes, such as the ability to bind peptides and the recognition and uptake by the alpha (2) macroglobulin receptor, the Applicants have discovered that α2M can be used in the methods described herein for immunotherapy against cancer and infectious disease. Alpha-2-macroglobulin can form complexes with antigens, which are taken up by antigen presenting cells ("APCs") via the alpha (2) macroglobulin receptor, also known as
30 LDL (low-density lipoprotein) Receptor-Related Protein ("LRP") or CD91. Thus, the invention provides methods and compositions for using specific α2M-antigenic molecule complexes for targeting an immune response against immune disorders, proliferative disorders, and infectious diseases.

The invention encompasses complexes of alpha (2) macroglobulin noncovalently
35 associated antigenic molecules, recombinant cells that express the complexes of α2M associated with antigenic molecules, and antibodies and other molecules that specifically

recognize α 2M-antigenic molecule complexes. The invention also provides methods for using these compositions in the diagnosis and treatment of immune disorders, proliferative disorders, and infectious diseases.

5 As used herein, an alpha (2) macroglobulin is associated with an antigenic molecule is bound to the antigenic molecule by a covalent or noncovalent bond. A covalent bond can be a peptide bond or a thioester linkage, for example. Thus, fusion proteins between alpha (2) macroglobulin and an antigenic molecule are within the scope of the invention.

10 The invention provides a pharmaceutical composition comprising an amount of a molecular complex effective for treatment or prevention of an infectious disease or cancer, and a pharmaceutically acceptable carrier, said molecular complex comprising an alpha (2) macroglobulin polypeptide noncovalently associated with an antigenic molecule which displays the antigenicity of an antigen of an infectious agent or an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type. As used herein a cell type of a cancer cell, refers to the cell type of the tissue of origin, *e.g.*, breast, lung, ovarian. In one embodiment, the antigenic molecule displays the antigenicity of an antigen of an infectious agent. In another embodiment, the antigenic molecule displays the antigenicity of an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type. In another embodiment, the antigenic molecule is a tumor specific antigen or a tumor-associated antigen. In another embodiment, the antigenic molecule displays the antigenicity of an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.

25 In another embodiment, the molecular complex effective for treatment or prevention of an infectious disease or cancer, comprising the alpha (2) macroglobulin polypeptide noncovalently associated with the antigenic molecule is purified. In particular, the term "purified" molecular complexes refer to complexes which are at least 65%, 75%, 80%, 85%, 90%, 95%, 98% or 100% noncovalent complexes of the alpha (2) macroglobulin polypeptide and the antigenic molecule. In another embodiment, the purified molecular complex comprising an alpha (2) macroglobulin polypeptide associated with an antigenic molecule of an infectious agent or an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.

30 The invention further provides a purified population of molecular complexes in which at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% of the complexes comprise an alpha (2) macroglobulin noncovalently associated with an antigenic molecule. Also provided by the invention is a purified population of molecular complexes purified from a recombinant cell in which at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% of the complexes comprise an alpha (2) macroglobulin noncovalently associated with an antigenic molecule.

The invention also provides a recombinant cell infected with a pathogen and transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin polypeptide, which alpha (2) macroglobulin polypeptide associates with an antigenic molecule, when said antigenic molecule is present, to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule. The invention provides a recombinant cancer cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin polypeptide, which alpha (2) macroglobulin polypeptide associates with an antigenic molecule, when said antigen is present, to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule. In another embodiment, the invention provides a recombinant cell transformed with (i) a first nucleic acid comprising a first nucleotide sequence that is operably linked to a first promoter and encodes an alpha (2) macroglobulin polypeptide, and (ii) a second nucleic acid comprising a second nucleotide sequence that is operably linked to a second promoter and encodes an antigenic molecule, such that the alpha (2) macroglobulin polypeptide and the antigenic molecule are expressed within the cell and associate with each other to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule. In various embodiments, the recombinant cells are human cells. In various embodiments, the pharmaceutical composition comprises a recombinant cell and a pharmaceutically acceptable carrier.

In one embodiment, a method is provided for preparing a complex of an alpha (2) macroglobulin polypeptide noncovalently associated with an antigenic molecule, said alpha (2) macroglobulin polypeptide comprising: (a) culturing a cell transformed with a nucleic acid comprising a nucleotide sequence encoding the alpha (2) macroglobulin polypeptide, under conditions such that said alpha (2) macroglobulin polypeptide is expressed by the cells and associates with an antigenic molecule of the cell; and (b) recovering a population of complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules from the cells.

The invention further provides a method for preparing an alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules derived from one or more antigens of an infectious agent, comprising: culturing infected cells, transformed with a nucleic acid comprising a nucleotide sequence encoding the alpha (2) macroglobulin polypeptide, or fragment, analog, or variant thereof, and operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the cells and associates with peptides of the cells; and (b) recovering from the cells a population of complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with peptides derived from the infectious agent. In one embodiment, the method further

comprises purifying the complexes. In another embodiment, the method further comprising purifying the complexes by affinity chromatography.

5 The invention further provides a method of treating or preventing an infectious disease in an individual having an infectious disease comprising administering to the individual one or more immunogenic complexes of an alpha (2) macroglobulin polypeptide noncovalently associated with a first antigenic molecule, wherein the first antigenic molecule displays the antigenicity of an antigen of an infectious agent of the infectious disease. In another embodiment, the method further comprises, before, concurrently or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second complex of alpha (2) macroglobulin polypeptide noncovalently associated with a second antigenic molecule, said second antigenic molecule displaying the antigenicity of a second antigenic molecule of said infectious agent.

15 The invention further provides a method of treating or preventing an infectious disease in a subject having an infectious disease comprising: a) culturing an infected cell transformed with a nucleic acid comprising a nucleotide sequence encoding an alpha (2) macroglobulin polypeptide, said infected cell displaying the antigenicity of an antigen of an infectious agent of the infectious disease, said nucleotide sequence being operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the infected cells and associates with antigenic molecules of the cell; b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules from the infected cell; and c) administering to the subject an amount of the recovered complexes effective to treat or prevent the infectious disease. In one embodiment, the method further comprises, prior to step (a), the step of obtaining infected cells from the subject and transforming the infected cells with the nucleic acid. In another embodiment, the method further comprises, prior to step (a), the step of obtaining the infected cell from one or more individuals and transforming the infected cells with the nucleic acid, said one or more individuals being different from the subject and having the same type of infectious disease as the subject.

25 The invention further provides a method of treating or preventing an infectious disease in a subject having an infectious disease comprising: a) culturing a recombinant cell transformed with (i) a first nucleic acid encoding an alpha (2) macroglobulin polypeptide and (ii) a second nucleic acid encoding an antigenic molecule displaying the antigenicity of an antigen of an infectious agent of the infectious disease; b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with the antigen; and c) administering to the subject an amount of the recovered complexes effective to treat or prevent the infectious disease. In various embodiments, the infectious disease is caused by

an infectious agent selected from the group consisting of a virus, a bacterium, a fungus, and a parasite.

Also provide by the invention is a method of treating or preventing cancer in an individual having a type of cancer or in whom prevention of a type of cancer is desired comprising administering to the individual an immunogenic complex of an alpha (2) 5
macroglobulin polypeptide noncovalently associated with a first antigenic molecule, wherein either (a) the first antigenic molecule displays antigenicity of said type of cancer or a metastasis thereof; or (b) the complex is obtained by recovering complexes from said type of cancer cells or a metastasis thereof that recombinantly express the alpha (2) macroglobulin polypeptide. In one embodiment, this method further comprises, before, concurrently or 10
after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second complex of an alpha (2) macroglobulin noncovalently associated with a second antigenic molecule, said second antigenic molecule displaying the antigenicity of an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell 15
type. an infectious agent of the infectious disease. In another embodiment, the first antigenic molecule is an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.

In one embodiment, a method is provided for treating or preventing cancer in a subject having a type of cancer or in whom prevention of a type of cancer is desired 20
comprising: a) culturing a cancer cell of said type transformed with a nucleic acid comprising a nucleotide sequence encoding an alpha (2) macroglobulin polypeptide said nucleotide sequence being operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the cancer cell and associates with at least one antigenic molecule of the cell; b) recovering complexes of the alpha (2) macroglobulin 25
polypeptide noncovalently associated with at least one antigenic molecule from the cancer cell; and c) administering to the subject an amount of the recovered complexes effective to treat or prevent cancer. In one embodiment, the method further comprises, prior to step (a), the step of obtaining cancer cells from the subject and transforming the cancer cells with the nucleic acid. In another embodiment, the method further comprises, prior to step (a), the step 30
of obtaining cancer cells from one or more individuals and transforming the cancer cells with the nucleic acid, said one or more individuals being different from the subject and having the same type of cancer as the subject.

The invention further provides a method of treating or preventing cancer in a subject 35
having a type of cancer or in whom prevention of a type of cancer is desired comprising: a) culturing a recombinant cell transformed with (i) a first nucleic acid encoding an alpha (2) macroglobulin polypeptide, and (ii) a second nucleic acid encoding an antigenic molecule

displaying the antigenicity of an antigen of a cancer cell; b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with the antigen; and c) administering to the subject an amount of the recovered complexes effective to treat or prevent cancer.

5 As used herein a "type of cancer" refers to *e.g.*, melanoma, breast cancer, renal carcinoma, or a metastasis thereof, where a metastasis refers to the same type of cancer as the cell of origin. In various embodiments, the cancer is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon
10 carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular
15 tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, multiple myeloma, Waldenström's
20 macroglobulinemia, and heavy chain disease.

The invention also encompasses a method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment an antibody specific for alpha (2) macroglobulin. In one embodiment, the antibody is purified.

25

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A-D . Identification of an 80 kDa polypeptide as a putative gp96 receptor. A. Confocal microscopy of re-presentation-competent RAW264.7 cells stained with gp96-FITC and B. with albumin-FITC. C. SDS-PAGE analysis of detergent extracts of plasma
30 membranes from surface biotinylated RAW264.7 (re-presentation-competent) or P815 cells (representation-incompetent) eluted from gp96 or albumin-Sepharose (SA) columns and stained with silver stain (top) or avidin-peroxidase (bottom). D. gp96-SASD-I¹²⁵ was cross-linked to live peritoneal macrophages (MO) or P815 cells, and the cell lysates examined by SDS-PAGE and autoradiography. Various components were omitted as
35 controls, as indicated.

5 **FIG. 2A-B.** Anti-p80 antiserum detects an 80 kDa molecule and inhibits re-presentation of gp96-chaperoned AHI peptide by macrophage. **A.** Pre-immune and immune sera were used to probe blots of plasma membrane extracts of RAW264.7, peritoneal macrophages (both cell types re-presentation-competent), or P815 cells. **B.** Re-presentation of gp96-chaperoned peptide AHI. Sera were added at the final dilution indicated. The solid cross indicates the level of T cell stimulation when the APCs were pulsed directly with the AHI peptide. The open cross indicates the corresponding value with unpulsed APCs.

10 **FIG. 3A-C.** Protein microsequencing of the 80 kDa protein. **A.** Analysis of a single tryptic (GALHIYHQR) peptide by tandem- mass spectrometry. All possible b- and y-ion series together with identified b-ion series (red) and y-ion series (blue) are shown. **B.** Collision-induced dissociation (CID) spectrum of this peptide is shown. **C.** Four identified peptides from the $\alpha 2M$ receptor, peptide mass, and sequence are shown.

15 **FIG. 4.** $\alpha 2$ -Macroglobulin inhibits re-presentation of gp96-chaperoned AHI peptide by macrophage. The solid cross indicates the level of T cell stimulation when the APCs were pulsed directly with the AHI peptide. The open cross indicates the corresponding value with unpulsed APCs.

20 **FIG. 5.** $\alpha 2M$ receptor is a sensor of necrotic cell death due to its ability to detect extracellular gp96. Conversely, receptors (psR) for phosphatidyl serine (ps) detect apoptotic cell death.

25 **FIG. 6A-B.** **A.** The mouse $\alpha 2MR$ cDNA (SEQ ID NO:1) and predicted open reading frame of murine $\alpha 2MR$ protein (Genbank accession no. CAA47817). **B.** The murine $\alpha 2M$ protein (SEQ ID NO:2), with residues identified by microsequencing an 80 kDa, gp96-interacting fragment of the receptor highlighted in bold.

30 **FIG. 7A-B.** **A.** Amino acid sequence of $\alpha 2M$ (SEQ ID NO: 3). **B.** Nucleotide sequence of $\alpha 2M$ (SEQ ID NO: 4). The 138 amino acid sequence (SEQ ID NO.: 5) of the receptor binding domain from $\alpha 2M$ is underlined.

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5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions and methods for alpha (2) macroglobulin ("α2M") vaccines for use in immunotherapy. The invention is based, in part, on the Applicant's discovery that α2M blocks uptake of heat shock proteins by antigen presenting cells. In particular, the invention provides complexes of α2M associated with antigenic molecules, which are recognized by the alpha (2) macroglobulin receptor on antigen presenting cells ("APCs"), and are presented by such cells to the immune system. Thus, the invention provides methods and compositions for using specific α2M-antigenic molecule complexes for targeting an immune response against immune disorders, proliferative disorders, and infectious diseases.

The human plasma protein alpha (2) macroglobulin is a 720 kDa homotetrameric proteinase inhibitor primarily known as proteinase inhibitor and plasma and inflammatory fluid proteinase scavenger molecule (for review see Chu and Pizzo, 1994, Lab. Invest. 71:792). During proteolytic activation of α2M, non-proteolytic ligands can become incorporated, covalently and noncovalently, to the activated thioesters (see Osada *et al.*, 1987, Biochem. Biophys. Res. Comm. 146:26-31; Osada *et al.*, 1988, Biochem. Biophys. Res. Comm. 150:883-889; Chu and Pizzo, 1993, J. Immunology 150: 48-58; Chu *et al.*, 1994, 152:1538-1545; Mitsuda *et al.*, 1993, Biochem. Biophys. Res. Comm. 191:1326-1331). As described herein, when complexes formed between α2M and an antigenic molecule having the antigenicity of a cancer cell antigen or of a pathogen, such α2M-antigenic molecule complexes can be used to stimulate a cytotoxic T cell response directed against the α2M incorporated antigen. Such complexes can be used as immunotherapeutic agents to treat cancer and infectious diseases.

Described in detailed hereinbelow are methods and compositions for use in preparation and delivery of such therapeutic α2M-antigenic molecule complexes. The invention encompasses complexes of alpha (2) macroglobulin associated antigenic molecules, antigenic cells that express the α2M, and antibodies and other molecules that specifically recognize α2M-antigenic molecule complexes. The invention also relates to methods for using these compositions in the diagnosis and treatment of immune disorders, proliferative disorders, and infectious diseases.

5.1 COMPOSITIONS OF THE INVENTION

The present invention provides compositions that can be used in immunotherapy against proliferative disorders, infectious diseases, and immune disorders. Such compositions include antibodies that specifically recognize α2M complexes, isolated

antigenic cells that express $\alpha 2M$ complexes, and recombinant cells that contain recombinant $\alpha 2M$ and sequences encoding antigenic molecules.

5 It is contemplated that the definition of $\alpha 2M$, as used herein, embraces other polypeptide fragments, analogs, and variants of $\alpha 2M$ having at least 35% to 55%, preferably 55% to 75%, and most preferably 75% to 85% amino acid identity with $\alpha 2M$, and is capable
10 of forming a complex with an antigenic molecule, which complex is capable of being taken up by an antigen presenting cell and eliciting an immune response against the antigenic molecule. The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a
15 mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al., 1990, J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST
20 program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which
25 detects distant relationships between molecules (Altschul *et al.*, 1997, *supra*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller,
30 1988, CABIOS 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

30 The immunogenic $\alpha 2M$ -antigenic molecule complexes of the invention may include any complex containing an $\alpha 2M$ and an antigenic peptide that is capable of inducing an immune response in a mammal.

$\alpha 2M$ and/or antigenic molecules can be purified from natural sources, chemically synthesized, or recombinantly produced.

35

5.1.1. α 2M POLYPEPTIDES

The alpha (2) macroglobulin complex of the invention is comprised of an alpha (2) macroglobulin polypeptide associated with an antigenic peptide. Alpha (2) macroglobulin polypeptides may be produced by recombinant DNA techniques, synthetic methods, or by enzymatic or chemical cleavage of native α 2M polypeptides. Described herein are methods for producing such α 2M polypeptides..

5.1.1.1 ISOLATION OF α 2M GENE SEQUENCES

In various aspects, the invention relates to compositions comprising amino acid sequences of α 2M, and fragments, derivatives, analogs, and variants thereof. Nucleic acids encoding α 2M are provided, as well as nucleic acids complementary to and capable of hybridizing to such nucleic acids.

Any eukaryotic cell may serve as the nucleic acid source for obtaining the coding region of an α 2M gene. Nucleic acid sequences encoding α 2M can be isolated from vertebrate, mammalian, as well as primate sources, including humans.

Amino acid sequences and nucleotide sequences of naturally occurring α 2M polypeptides are generally available in sequence databases, such as GenBank. Non-limiting examples of α 2M sequences that can be used for preparation of the α 2M polypeptides of the invention are as follows: Genbank Accession Nos. M11313, P01023, AAA51551; Kan *et al.*, 1985, Proc. Nat. Acad. Sci. 82: 2282-2286. Due to the degeneracy of the genetic code, the term " α 2M gene", as used herein, refers not only to the naturally occurring nucleotide sequence but also encompasses all the other degenerate DNA sequences that encode an α 2M polypeptide. Computer programs, such as Entrez, can be used to browse the database, and retrieve any amino acid sequence and genetic sequence data of interest by accession number. These databases can also be searched to identify sequences with various degrees of similarities to a query sequence using programs, such as FASTA and BLAST, which rank the similar sequences by alignment scores and statistics. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res.25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Altschul *et al.*, 1997, *supra*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see <http://www.ncbi.nlm.nih.gov>).

The DNA may be obtained by standard procedures known in the art by DNA amplification or molecular cloning directly from a tissue, cell culture, or cloned DNA (*e.g.*, a DNA "library"). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the $\alpha 2M$ gene should be cloned into a suitable vector for propagation of the gene.

In a preferred embodiment, DNA can be amplified from genomic or cDNA by polymerase chain reaction (PCR) amplification using primers designed from the known sequence of a related or homologous $\alpha 2M$. PCR is used to amplify the desired sequence in DNA clone or a genomic or cDNA library, prior to selection. PCR can be carried out, *e.g.*, by use of a thermal cycler and Taq polymerase (sold under the trademark GENE AMP). The DNA being amplified can include cDNA or genomic DNA from any species. Oligonucleotide primers representing known nucleic acid sequences of related HSPs can be used as primers in PCR. In a preferred aspect, the oligonucleotide primers represent at least part of the $\alpha 2M$ gene that is highly conserved between $\alpha 2M$ genes of different species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the known $\alpha 2M$ nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred. For same species hybridization, moderately stringent conditions are preferred. After successful amplification, the sequence encoding an $\alpha 2M$ may be cloned and sequenced. If the size of the coding region of the $\alpha 2M$ gene being amplified is too large to be amplified in a single PCR, several PCR covering the entire gene, preferably with overlapping regions, may be carried out, and the products of the PCR ligated together to form the entire coding sequence. Alternatively, if a segment of an $\alpha 2M$ gene is amplified, that segment may be cloned, and utilized as a probe to isolate a complete cDNA or genomic clone.

In another embodiment, for the molecular cloning of an $\alpha 2M$ gene from genomic DNA, DNA fragments are generated to form a genomic library. Since some of the sequences encoding related $\alpha 2Ms$ are available and can be purified and labeled, the cloned DNA fragments in the genomic DNA library may be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available.

Alternatives to isolating the $\alpha 2M$ genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes $\alpha 2M$. For example, RNA for cDNA cloning of the $\alpha 2M$ gene can be isolated from cells which express $\alpha 2M$. A cDNA library may be generated by methods known in the art and screened by methods, such as those disclosed for screening a genomic DNA library. If an antibody to $\alpha 2M$ is available, $\alpha 2M$ may be identified by binding of labeled antibody to the putatively $\alpha 2M$ synthesizing clones.

Other specific embodiments for the cloning of a nucleotide sequence encoding an $\alpha 2M$, are presented as examples but not by way of limitation, as follows:

In a specific embodiment, nucleotide sequences encoding $\alpha 2M$ proteins within a family can be identified and obtained by hybridization with a probe comprising nucleotide sequence encoding $\alpha 2M$ under conditions of low to medium stringency.

By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792). Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μ g/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μ g/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 $\times 10^6$ cpm ^{32}P -labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

An $\alpha 2M$ gene fragment can be inserted into an appropriate cloning vector and introduced into host cells so that many copies of the gene sequence are generated. A large number of vector-host systems known in the art may be used such as, but not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene).

Any technique for mutagenesis known in the art can be used to modify individual nucleotides in a DNA sequence, for purpose of making amino acid substitution(s) in the expressed peptide sequence, or for creating/deleting restriction sites to facilitate further manipulations. Such techniques include but are not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson *et al.*, 1978, J. Biol. Chem 253:6551), oligonucleotide-directed mutagenesis (Smith, 1985, Ann. Rev. Genet. 19:423-463; Hill *et al.*,



1987, Methods Enzymol. 155:558-568), PCR-based overlap extension (Ho *et al.*, 1989, Gene 77:51-59), PCR-based megaprimer mutagenesis (Sarkar *et al.*, 1990, Biotechniques, 8:404-407), *etc.* Modifications can be confirmed by double stranded dideoxy DNA sequencing.

5 The polymerase chain reaction (PCR) is commonly used for obtaining genes or gene fragments of interest. For example, a nucleotide sequence encoding $\alpha 2M$ polypeptide of any desired length can be generated using PCR primers that flank the nucleotide sequence encoding $\alpha 2M$, or the peptide-binding domain thereof. Alternatively, an $\alpha 2M$ gene sequence can be cleaved at appropriate sites with restriction endonuclease(s) if such sites are available, releasing a fragment of DNA encoding $\alpha 2M$, or the peptide-binding domain thereof. If
10 convenient restriction sites are not available, they may be created in the appropriate positions by site-directed mutagenesis and/or DNA amplification methods known in the art (see, for example, Shankarappa *et al.*, 1992, PCR Method Appl. 1:277-278). The DNA fragment that encodes $\alpha 2M$, or the peptide-binding domain thereof, is then isolated, and ligated into an appropriate expression vector, care being taken to ensure that the proper translation reading
15 frame is maintained.

Alpha (2) macroglobulin polypeptides of the invention may be expressed as fusion proteins to facilitate recovery and purification from the cells in which they are expressed. For example, an $\alpha 2M$ polypeptide may contain a signal sequence leader peptide to direct its translocation across the ER membrane for secretion into culture medium. Further, an $\alpha 2M$
20 polypeptide may contain an affinity label, such as a affinity label, fused to any portion of the $\alpha 2M$ polypeptide not involved in binding antigenic peptide, such as for example, the carboxyl terminal. The affinity label can be used to facilitate purification of the protein, by binding to an affinity partner molecule.

Various methods for production of such fusion proteins are well known in the art. The manipulations which result in their production can occur at the gene or protein level,
25 preferably at the gene level. For example, the cloned coding region of an $\alpha 2M$ polypeptide may be modified by any of numerous recombinant DNA methods known in the art (Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Ausubel *et al.*, in Chapter 8 of Current
30 Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York). It will be apparent from the following discussion that substitutions, deletions, insertions, or any combination thereof are introduced or combined to arrive at a final nucleotide sequence encoding an $\alpha 2M$ polypeptide.

In various embodiments, fusion proteins comprising the $\alpha 2M$ polypeptide may be
35 made using recombinant DNA techniques. For example, a recombinant gene encoding an $\alpha 2M$ polypeptide may be constructed by introducing an $\alpha 2M$ gene fragment in the proper reading frame into a vector containing the sequence of an affinity label, such that the $\alpha 2M$

polypeptide is expressed as a peptide-tagged fusion protein. Affinity labels, which may be recognized by specific binding partners, may be used for affinity purification of the $\alpha 2M$ polypeptide.

5 In a preferred embodiment, the affinity label is fused at its amino terminal to the carboxyl terminal of $\alpha 2M$. The precise site at which the fusion is made in the carboxyl terminal is not critical. The optimal site can be determined by routine experimentation.

A variety of affinity labels known in the art may be used, such as, but not limited to, the immunoglobulin constant regions, polyhistidine sequence (Petty, 1996, Metal-chelate affinity chromatography, in Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel *et al.*, Greene Publish. Assoc. & Wiley Interscience), glutathione S-transferase (GST; Smith, 10 1993, Methods Mol. Cell Bio. 4:220-229), the *E. coli* maltose binding protein (Guan *et al.*, 1987, Gene 67:21-30), and various cellulose binding domains (U.S. Patent Nos. 5,496,934; 5,202,247; 5,137,819; Tomme *et al.*, 1994, Protein Eng. 7:117-123), etc. Other affinity labels may impart fluorescent properties to an $\alpha 2M$ polypeptide, *e.g.*, portions of green 15 fluorescent protein and the like. Other possible affinity labels are short amino acid sequences to which monoclonal antibodies are available, such as but not limited to the following well known examples, the FLAG epitope, the myc epitope at amino acids 408-439, the influenza virus hemagglutinin (HA) epitope. Other affinity labels are recognized by specific binding partners and thus facilitate isolation by affinity binding to the binding partner which can be immobilized onto a solid support. Some affinity labels may afford the $\alpha 2M$ polypeptide 20 novel structural properties, such as the ability to form multimers. Dimerization of an $\alpha 2M$ polypeptide with a bound peptide may increase avidity of interaction between the $\alpha 2M$ polypeptide and its partner in the course of antigen presentation. These affinity labels are usually derived from proteins that normally exist as homopolymers. Affinity labels such as the extracellular domains of CD8 (Shiue *et al.*, 1988, J. Exp. Med. 168:1993-2005), or CD28 25 (Lee *et al.*, 1990, J. Immunol. 145:344-352), or portions of the immunoglobulin molecule containing sites for interchain disulfide bonds, could lead to the formation of multimers. As will be appreciated by those skilled in the art, many methods can be used to obtain the coding region of the above-mentioned affinity labels, including but not limited to, DNA cloning, DNA amplification, and synthetic methods. Some of the affinity labels and reagents for their 30 detection and isolation are available commercially.

A preferred affinity label is a non-variable portion of the immunoglobulin molecule. Typically, such portions comprise at least a functionally operative CH2 and CH3 domain of the constant region of an immunoglobulin heavy chain. Fusions are also made using the 35 carboxyl terminus of the Fc portion of a constant domain, or a region immediately amino-terminal to the CH1 of the heavy or light chain. Suitable immunoglobulin-based affinity label may be obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD, or IgM, but

preferably IgG1. Preferably, a human immunoglobulin is used when the $\alpha 2M$ polypeptide is intended for *in vivo* use for humans. Many DNA encoding immunoglobulin light or heavy chain constant regions is known or readily available from cDNA libraries. See, for example, Adams *et al.*, Biochemistry, 1980; 19:2711-2719; Gough *et al.*, 1980, Biochemistry, 19:2702-2710; Dolby *et al.*, 1980, Proc. Natl. Acad. Sci. U.S.A., 77:6027-6031; Rice *et al.*, 5 1982, Proc. Natl. Acad. Sci. U.S.A., 79:7862-7865; Falkner *et al.*, 1982, Nature, 298:286-288; and Morrison *et al.*, 1984, Ann. Rev. Immunol, 2:239-256. Because many immunological reagents and labeling systems are available for the detection of immunoglobulins, the $\alpha 2M$ polypeptide-Ig fusion protein can readily be detected and 10 quantified by a variety of immunological techniques known in the art, such as the use of enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, fluorescence activated cell sorting (FACS), etc. Similarly, if the affinity label is an epitope with readily available antibodies, such reagents can be used with the techniques mentioned above to detect, quantitate, and isolate the $\alpha 2M$ polypeptide containing the affinity label. In many instances, 15 there is no need to develop specific antibodies to the $\alpha 2M$ polypeptide.

A particularly preferred embodiment is a fusion of an $\alpha 2M$ polypeptide to the hinge, the CH2 and CH3 domains of human immunoglobulin G-1 (IgG-1; see Bowen *et al.*, 1996, J. Immunol. 156:442-49). This hinge region contains three cysteine residues which are normally involved in disulfide bonding with other cysteines in the Ig molecule. Since none 20 of the cysteines are required for the peptide to function as a tag, one or more of these cysteine residues may optionally be substituted by another amino acid residue, such as for example, serine.

Various leader sequences known in the art can be used for the efficient secretion of $\alpha 2M$ polypeptide from bacterial and mammalian cells (von Heijne, 1985, J. Mol. Biol. 184:99-105). Leader peptides are selected based on the intended host cell, and may include 25 bacterial, yeast, viral, animal, and mammalian sequences. For example, the herpes virus glycoprotein D leader peptide is suitable for use in a variety of mammalian cells. A preferred leader peptide for use in mammalian cells can be obtained from the V-J2-C region of the mouse immunoglobulin kappa chain (Bernard *et al.*, 1981, Proc. Natl. Acad. Sci. 78:5812-5816). Preferred leader sequences for targeting $\alpha 2M$ polypeptide expression in bacterial cells 30 include, but are not limited to, the leader sequences of the *E. coli* proteins OmpA (Hobom *et al.*, 1995, Dev. Biol. Stand. 84:255-262), Pho A (Oka *et al.*, 1985, Proc. Natl. Acad. Sci. 82:7212-16), OmpT (Johnson *et al.*, 1996, Protein Expression 7:104-113), LamB and OmpF (Hoffman & Wright, 1985, Proc. Natl. Acad. Sci. USA 82:5107-5111), β -lactamase (Kadonaga *et al.*, 1984, J. Biol. Chem. 259:2149-54), enterotoxins (Morioka-Fujimoto *et al.*, 35 1991, J. Biol. Chem. 266:1728-32), and the *Staphylococcus aureus* protein A (Abrahmsen *et al.*, 1986, Nucleic Acids Res. 14:7487-7500), and the *B. subtilis* endoglucanase (Lo *et al.*,

Appl. Environ. Microbiol. 54:2287-2292), as well as artificial and synthetic signal sequences (MacIntyre *et al.*, 1990, Mol. Gen. Genet. 221:466-74; Kaiser *et al.*, 1987, Science, 235:312-317).

5 DNA sequences encoding a desired affinity label or leader peptide, which may be readily obtained from libraries, produced synthetically, or may be available from commercial suppliers, are suitable for the practice of this invention. Such methods are well known in the art.

5.1.1.2 RECOMBINANT EXPRESSION

10 In various embodiments of the invention, sequences encoding an $\alpha 2M$ polypeptide are inserted into an expression vector for propagation and expression in recombinant cells.

An expression construct, as used herein, refers to a nucleotide sequence encoding an $\alpha 2M$ polypeptide operably associated with one or more regulatory regions which allows expression of the $\alpha 2M$ polypeptide in an appropriate host cell. "Operably-associated" refers to an association in which the regulatory regions and the $\alpha 2M$ polypeptide sequence to be
15 expressed are joined and positioned in such a way as to permit transcription, and ultimately, translation.

Vectors based on *E. coli* are the most popular and versatile systems for high level expression of foreign proteins (Makrides, 1996, Microbiol Rev, 60:512-538). Non-limiting examples of regulatory regions that can be used for expression in *E. coli* may include but not
20 limited to *lac*, *trp*, *lpp*, *phoA*, *recA*, *tac*, λP_L , and phage T3 and T7 promoters (Makrides, 1996, Microbiol Rev, 60:512-538). Non-limiting examples of prokaryotic expression vectors may include the λ gt vector series such as λ gt11 (Huynh *et al.*, 1984 in "DNA Cloning Techniques", Vol. I: A Practical Approach (D. Glover, ed.), pp. 49-78, IRL Press, Oxford),
25 and the pET vector series (Studier *et al.*, 1990, Methods Enzymol., 185:60-89). However, a potential drawback of a prokaryotic host-vector system is the inability to perform many of the post-translational processing events of mammalian cells. Thus, an eukaryotic host-vector system is preferred, a mammalian host-vector system is more preferred, and a human host-vector system is the most preferred.

30 The regulatory regions necessary for transcription of the $\alpha 2M$ polypeptide can be provided by the expression vector. A translation initiation codon (ATG) may also be provided to express a nucleotide sequence encoding an $\alpha 2M$ polypeptide that lacks an initiation codon. In a compatible host-construct system, cellular proteins required for transcription, such as RNA polymerase and transcription factors, will bind to the regulatory
35 regions on the expression construct to effect transcription of the $\alpha 2M$ polypeptide sequence in the host organism. The precise nature of the regulatory regions needed for gene expression may vary from host cell to host cell. Generally, a promoter is required which is

capable of binding RNA polymerase to initiate the transcription of an operably-associated nucleic acid sequence. Such regulatory regions may include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, the cap site, a CAAT box, and the like. The non-coding region 3' to the coding sequence may contain transcriptional termination regulatory sequences, such as terminators and polyadenylation sites.

Both constitutive and inducible regulatory regions may be used for expression of the $\alpha 2M$ polypeptide. It may be desirable to use inducible promoters when the conditions optimal for growth of the recombinant cells and the conditions for high level expression of the $\alpha 2M$ polypeptide are different. Examples of useful regulatory regions are provided in the next section below.

For expression of $\alpha 2M$ polypeptides in mammalian host cells, a variety of regulatory regions can be used, for example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. Inducible promoters that may be useful in mammalian cells include but are not limited to those associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the β -interferon gene, and the $\alpha 2M70$ gene (Williams *et al.*, 1989, Cancer Res. 49:2735-42 ; Taylor *et al.*, 1990, Mol. Cell Biol., 10:165-75).

The following animal regulatory regions, which exhibit tissue specificity and have been utilized in transgenic animals, can also be used in tumor cells of a particular tissue type: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adames *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-

286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378).

The efficiency of expression of the $\alpha 2M$ polypeptide in a host cell may be enhanced by the inclusion of appropriate transcription enhancer elements in the expression vector, such as those found in SV40 virus, Hepatitis B virus, cytomegalovirus, immunoglobulin genes, metallothionein, β -actin (see Bittner *et al.*, 1987, Methods in Enzymol. 153:516-544; Gorman, 1990, Curr. Op. in Biotechnol. 1:36-47).

The expression vector may also contain sequences that permit maintenance and replication of the vector in more than one type of host cell, or integration of the vector into the host chromosome. Such sequences may include but are not limited to replication origins, autonomously replicating sequences (ARS), centromere DNA, and telomere DNA. It may also be advantageous to use shuttle vectors that can be replicated and maintained in at least two types of host cells.

In addition, the expression vector may contain selectable or screenable marker genes for initially isolating or identifying host cells that contain DNA encoding an $\alpha 2M$ polypeptide. For long term, high yield production of $\alpha 2M$ polypeptide-antigenic molecule complexes, stable expression in mammalian cells is preferred. A number of selection systems may be used for mammalian cells, including, but not limited, to the Herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalski and Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, Cell 22:817) genes can be employed in *tk*, *hgp^r* or *ap^r* cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dihydrofolate reductase (*dhfr*), which confers resistance to methotrexate (Wigler *et al.*, 1980, Natl. Acad. Sci. USA 77:3567; O'Hare *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neomycin phosphotransferase (*neo*), which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, 1981, J. Mol. Biol. 150:1); and hygromycin phosphotransferase (*hyg*), which confers resistance to hygromycin (Santerre *et al.*, 1984, Gene 30:147). Other selectable markers, such as but not limited to histidinol and ZeocinTM can also be used.

In order to insert the $\alpha 2M$ polypeptide DNA sequence into the cloning site of a vector, DNA sequences with regulatory functions, such as promoters, must be attached to DNA sequences encoding the $\alpha 2M$ peptide-binding region. To do this, linkers or adapters providing the appropriate compatible restriction sites may be ligated to the ends of cDNA or synthetic DNA encoding an $\alpha 2M$ polypeptide, by techniques well known in the art (Wu *et al.*, 1987, Methods in Enzymol 152:343-349). Cleavage with a restriction enzyme can be followed by modification to create blunt ends by digesting back or filling in single-stranded

DNA termini before ligation. Alternatively, a desired restriction enzyme site can be introduced into a fragment of DNA by amplification of the DNA by use of PCR with primers containing the desired restriction enzyme site.

5 An expression construct comprising an $\alpha 2M$ polypeptide sequence operably associated with regulatory regions can be directly introduced into appropriate host cells for expression and production of $\alpha 2M$ polypeptide-antigenic molecule complexes without further cloning (see, for example, U.S. Patent No. 5,580,859). The expression constructs may also contain DNA sequences that facilitate integration of the $\alpha 2M$ polypeptide sequence into the genome of the host cell, *e.g.*, via homologous recombination. In this instance, it is not necessary to employ an expression vector comprising a replication origin suitable for
10 appropriate host cells in order to propagate and express the $\alpha 2M$ polypeptide in the host cells.

Expression constructs containing cloned nucleotide sequence encoding $\alpha 2M$ polypeptides can be introduced into the host cell by a variety of techniques known in the art, including but not limited to, for prokaryotic cells, bacterial transformation (Hanahan, 1985,
15 in DNA Cloning, A Practical Approach, 1:109-136), and for eukaryotic cells, calcium phosphate mediated transfection (Wigler *et al.*, 1977, Cell 11:223-232), liposome-mediated transfection (Schaefer-Ridder *et al.*, 1982, Science 215:166-168), electroporation (Wolff *et al.*, 1987, Proc Natl Acad Sci 84:3344), and microinjection (Cappechi, 1980, Cell 22:479-488). Co-expression of an $\alpha 2M$ polypeptide and an antigenic molecule in the same host cell
20 can be achieved by essentially the same methods.

For long term, high yield production of properly processed $\alpha 2M$ polypeptides or $\alpha 2M$ polypeptide-antigenic molecule complexes, stable expression in mammalian cells is preferred. Cell lines that stably express $\alpha 2M$ polypeptides or $\alpha 2M$ polypeptide-antigenic molecule complexes may be engineered by using a vector that contains a selectable marker.
25 By way of example but not limitation, following the introduction of the expression constructs, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the expression construct confers resistance to the selection and optimally allows cells to stably integrate the expression construct into their chromosomes and to grow in culture and to be expanded into
30 cell lines. Such cells can be cultured for a long period of time while $\alpha 2M$ polypeptide is expressed continuously.

The recombinant cells may be cultured under standard conditions of temperature, incubation time, optical density, and media composition. Alternatively, a recombinant antigenic cells may be cultured under conditions emulating the nutritional and physiological
35 requirements of the cancer cell or infected cell. However, conditions for growth of recombinant cells may be different from those for expression of $\alpha 2M$ polypeptides and

antigenic proteins. Modified culture conditions and media may also be used to enhance production of $\alpha 2M$ -antigenic molecule complexes. Any techniques known in the art may be applied to establish the optimal conditions for producing $\alpha 2M$ polypeptide or $\alpha 2M$ polypeptide-antigenic molecule complexes.

5

5.1.1.3 PURIFICATION METHODS FOR RECOMBINANT $\alpha 2M$ POLYPEPTIDES

Generally, the $\alpha 2M$ polypeptides of the invention can be recovered and purified from recombinant cell cultures by known methods, including ammonium sulfate precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose
10 chromatography, immunoaffinity chromatography, hydroxyapatite chromatography, and lectin chromatography.

The invention provides methods for purification of recombinant $\alpha 2M$ polypeptides by affinity purification, based on the properties of the affinity label present on the $\alpha 2M$
15 polypeptide. One approach is based on specific molecular interactions between a tag and its binding partner. The other approach relies on the immunospecific binding of an antibody to an epitope present on the tag. The principle of affinity chromatography well known in the art is generally applicable to both of these approaches.

Described below are several methods based on specific molecular interactions of a
20 tag and its binding partner.

A method that is generally applicable to purifying recombinant $\alpha 2M$ s that are fused to the constant regions of immunoglobulin is protein A affinity chromatography, a technique that is well known in the art. Staphylococcus protein A is a 42 kD polypeptide that binds specifically to a region located between the second and third constant regions of heavy chain immunoglobulins. Because of the Fc domains of different classes, subclasses and species of
25 immunoglobulins, affinity of protein A for human Fc regions is strong, but may vary with other species. Subclasses that are less preferred include human IgG-3, and most rat subclasses. For certain subclasses, protein G (of Streptococci) may be used in place of protein A in the purification. Protein-A sepharose (Pharmacia or Biorad) is a commonly used solid phase for affinity purification of antibodies, and can be used essentially in the
30 same manner for the purification of $\alpha 2M$ polypeptide fused to an immunoglobulin Fc fragment. Secreted $\alpha 2M$ polypeptide present in cell supernatant binds specifically to protein A on the solid phase, while the contaminants are washed away. Bound $\alpha 2M$ polypeptide can be eluted by various buffer systems known in the art, including a succession of citrate, acetate and glycine-HCl buffers which gradually lowers the pH. This method is less
35 preferred if the recombinant cells also produce antibodies which will be copurified with the $\alpha 2M$ polypeptide. See, for example, Langone, 1982, J. Immunol. meth. 51:3; Wilchek *et al.*,

1982, Biochem. Intl. 4:629; Sjobring *et al.*, 1991, J. Biol. Chem. 26:399; page 617-618, in Antibodies A Laboratory Manual, edited by Harlow and Lane, Cold Spring Harbor laboratory, 1988.

Alternatively, a polyhistidine tag may be used, in which case, the $\alpha 2M$ polypeptide can be purified by metal chelate chromatography. The polyhistidine tag, usually a sequence of six histidines, has a high affinity for divalent metal ions, such as nickel ions (Ni^{2+}), which can be immobilized on a solid phase, such as nitrilotriacetic acid matrices. Polyhistidine has a well characterized affinity for Ni^{2+} -NTA-agarose, and can be eluted with either of two mild treatments: imidazole (0.1-0.2 M) will effectively compete with the resin for binding sites; or lowering the pH just below 6.0 will protonate the histidine side-chains and disrupt the binding. The purification method comprises loading the cell culture supernatant onto the Ni^{2+} -NTA-agarose column, washing the contaminants through, and eluting the $\alpha 2M$ polypeptide with imidazole or weak acid. Ni^{2+} -NTA-agarose can be obtained from commercial suppliers such as Sigma (St. Louis) and Qiagen. Antibodies that recognize the polyhistidine tag are also available which can be used to detect and quantify the $\alpha 2M$ polypeptide.

Another exemplary affinity label that can be used is the glutathione-S-transferase (GST) sequence, originally cloned from the helminth, *Schistosoma japonicum*. In general, an $\alpha 2M$ -GST fusion expressed in a prokaryotic host cell, such as *E. coli*, can be purified from the cell culture supernatant by absorption with glutathione agarose beads, followed by elution in the presence of free reduced glutathione at neutral pH. Denaturing conditions are not required at any stage during purification, and therefore, it may be desirable for use in the loading of immobilized $\alpha 2M$ polypeptides with antigenic peptides. Moreover, since GST is known to form dimers under certain conditions, dimeric $\alpha 2M$ polypeptides may be obtained. See, Smith, 1993, Methods Mol. Cell Bio. 4:220-229.

Another useful affinity label that can be used is the maltose binding protein (MBP) of *E. coli*, which is encoded by the *malE* gene. The secreted $\alpha 2M$ polypeptide-MBP present in the cell supernatant binds to amylose resin while contaminants are washed away. The bound $\alpha 2M$ polypeptide-MBP is eluted from the amylose resin by maltose. See, for example, Guan *et al.*, 1987, Gene 67:21-30.

The second approach for purifying $\alpha 2M$ polypeptide is applicable to affinity labels that contain an epitope for which polyclonal or monoclonal antibodies are available. Various methods known in the art for purification of protein by immunospecific binding, such as immunoaffinity chromatography, and immunoprecipitation, can be used. See, for example, Chapter 13 in "Antibodies A Laboratory Manual", 1988, Harlow and Lane, (eds.), Cold Spring Harbor Laboratory, N.Y. and Chapter 8, Sections I and II, in "Current Protocols in

Immunology", 1991, Coligan *et al.* (eds.), John Wiley, the disclosure of which are both incorporated by reference herein.

5 The embodiments described above may be used to recover and purify $\alpha 2M$ polypeptide-antigenic molecule complexes from the cell culture medium of mammalian cells, such as human cells expressing an $\alpha 2M$ polypeptide of the invention. The methods can
10 be adapted to perform medium and large scale purification of an $\alpha 2M$ polypeptide and/or $\alpha 2M$ -antigenic molecule complexes. Methods that do not require lowering pH or denaturing conditions are most preferred for purification of $\alpha 2M$ polypeptide-antigenic molecule complexes. The methods may be used to isolate $\alpha 2M$ polypeptides from eukaryotic cells, for
15 example, cancer cells, tissues, isolated cells, or immortalized eukaryote cell lines infected with an intracellular pathogen, or cells obtained from a subject infected with a pathogen.

5.1.1.4 HOST-VECTOR SYSTEMS

Described herein are systems of vectors and host cells that can be used for the
15 expression of $\alpha 2M$ polypeptides. A variety of expression vectors may be used in the present invention which include, but are not limited to, plasmids, cosmids, phage, phagemids, or modified viruses. Typically, such expression vectors comprise a functional origin of replication for propagation of the vector in an appropriate host cell, one or more restriction
20 endonuclease sites for insertion of the $\alpha 2M$ polypeptide gene sequence, and one or more selection markers. The expression vector must be used with a compatible host cell which may be derived from a prokaryotic or an eukaryotic organism including but not limited to bacteria, yeasts, insects, mammals, and humans.

Expression constructs and vectors are introduced into host cells for the purpose of producing an $\alpha 2M$ polypeptide. Any cell type that can produce $\alpha 2Ms$ and is compatible with
25 the expression vector may be used, including those that have been cultured *in vitro* or genetically engineered. Host cells may be obtained from normal or affected subjects, including healthy humans, cancer patients, and patients with an infectious disease, private laboratory deposits, public culture collections such as the American Type Culture Collection, or from commercial suppliers.

30 Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. A host cell may be chosen which modifies and processes the expressed gene products in a specific fashion similar to the way the recipient processes $\alpha 2Ms$. For the purpose of producing large amounts of $\alpha 2M$, it is preferable that the type of host cell used in the present invention has been used for expression
35 of heterologous genes, and is reasonably well characterized and developed for large-scale production processes. In a specific embodiment, the host cells are from the same patient to

whom $\alpha 2M$ polypeptide-antigenic molecule complexes or recombinant cells expressing $\alpha 2M$ polypeptide-antigenic molecule complexes are going to be administered. Otherwise said, the cells used to express the $\alpha 2M$ polypeptide and used subsequently to administer immunotherapy to a subject are autologous to the subject.

5 Preferred mammalian host cells include but are not limited to those derived from humans, monkeys and rodents, (see, for example, Kriegler M. in "Gene Transfer and Expression: A Laboratory Manual", New York, Freeman & Co. 1990), such as monkey kidney cell line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293, 293-EBNA, or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen. Virol., 36:59, 1977; baby hamster kidney cells (BHK, ATCC CCL 10); chinese hamster ovary-cells-DHFR (CHO, Urlaub and Chasin. Proc. Natl. Acad. Sci. 77; 4216, 10 1980); mouse sertoli cells (Mather, Biol. Reprod. 23:243-251, 1980); mouse fibroblast cells (NIH-3T3), monkey kidney cells (CV1 ATCC CCL 70); african green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 15 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor cells (MMT 060562, ATCC CCL51). Exemplary cancer cell types used for demonstrating the utility of recombinant cells (producing $\alpha 2M$ polypeptide-antigenic molecule complexes) as a cancer vaccine are provided as follows: mouse fibroblast cell line, NIH3T3, mouse Lewis lung carcinoma cell line, LLC, mouse mastocytoma cell 20 line, P815, mouse lymphoma cell line, EL4 and its ovalbumin transfectant, E.G7, mouse melanoma cell line, B16F10, mouse fibrosarcoma cell line, MC57, and human small cell lung carcinoma cell lines, SCLC#2 and SCLC#7.

A number of viral-based expression systems may also be utilized with mammalian cells to produce $\alpha 2M$ polypeptides. Vectors using DNA virus backbones have been derived 25 from simian virus 40 (SV40) (Hamer *et al.*, 1979, Cell 17:725), adenovirus (Van Doren *et al.*, 1984, Mol Cell Biol 4:1653), adeno-associated virus (McLaughlin *et al.*, 1988, J Virol 62:1963), and bovine papillomas virus (Zinn *et al.*, 1982, Proc Natl Acad Sci 79:4897). In cases where an adenovirus is used as an expression vector, the donor DNA sequence may be ligated to an adenovirus transcription/translation control region, *e.g.*, the late promoter and 30 tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing heterologous products in infected hosts (see *e.g.*, Logan and Shenk, 1984, Proc. Natl. Acad. Sci. (USA) 81:3655-3659).

35 Bovine papillomavirus (BPV) can infect many higher vertebrates, including man, and its DNA replicates as an episome. A number of shuttle vectors have been developed for

recombinant gene expression which exist as stable, multicopy (20-300 copies/cell) extrachromosomal elements in mammalian cells. Typically, these vectors contain a segment of BPV DNA (the entire genome or a 69% transforming fragment), a promoter with a broad host range, a polyadenylation signal, splice signals, a selectable marker, and "poisonless" plasmid sequences that allow the vector to be propagated in *E. coli*. Following construction and amplification in bacteria, the expression gene construct is transfected into cultured mammalian cells, for example, by the techniques of calcium phosphate coprecipitation or electroporation. For those host cells that do not manifest a transformed phenotype, selection of transformants is achieved by use of a dominant selectable marker, such as histidinol and G418 resistance. For example, BPV vectors such as pBCMGSNeo and pBCMGRHis may be used to express α 2M polypeptide sequences (Karasuyama *et al.*, Eur. J. Immunol. 18:97-104; Ohe *et al.*, Human Gene Therapy, 6:325-33) which may then be transfected into a diverse range of cell types for expression of the α 2M polypeptide.

Alternatively, the vaccinia 7.5K promoter may be used (see, *e.g.*, Mackett *et al.*, 1982, Proc. Natl. Acad. Sci. (USA) 79:7415-7419; Mackett *et al.*, 1984, J. Virol. 49:857-864; Panicali *et al.*, 1982, Proc. Natl. Acad. Sci. 79:4927-4931.) In cases where a human host cell is used, vectors based on the Epstein-Barr virus (EBV) origin (OriP) and EBV nuclear antigen 1 (EBNA-1; a trans-acting replication factor) may be used. Such vectors can be used with a broad range of human host cells, *e.g.*, EBO-pCD (Spickofsky *et al.*, 1990, DNA Prot Eng Tech 2:14-18), pDR2 and λ DR2 (available from Clontech Laboratories).

α 2M polypeptides may also be made with a retrovirus-based expression system. In contrast to transfection, retroviruses can efficiently infect and transfer genes to a wide range of cell types including, for example, primary hematopoietic cells. In retroviruses such as Moloney murine leukemia virus, most of the viral gene sequences can be removed and replaced with nucleic acid sequences encoding α 2M, while the missing viral functions can be supplied in *trans*. The host range for infection by a retroviral vector can also be manipulated by the choice of envelope used for vector packaging.

For example, a retroviral vector can comprise a 5' long terminal repeat (LTR), a 3' LTR, a packaging signal, a bacterial origin of replication, and a selectable marker. The α 2M polypeptide DNA is inserted into a position between the 5' LTR and 3' LTR, such that transcription from the 5' LTR promoter transcribes the cloned DNA. The 5' LTR comprises a promoter, including but not limited to an LTR promoter, an R region, a U5 region and a primer binding site, in that order. Nucleotide sequences of these LTR elements are well known in the art. A heterologous promoter as well as multiple drug selection markers may also be included in the expression vector to facilitate selection of infected cells (see McLauchlin *et al.*, 1990, Prog. Nucleic Acid Res. and Molec. Biol. 38:91-135; Morgenstern *et al.*, 1990, Nucleic Acid Res. 18:3587-3596; Chouluka *et al.*, 1996, J. Virol 70:1792-1798;

Boesen *et al.*, 1994, *Biotherapy* 6:291-302; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114).

Other useful eukaryotic host-vector system may include yeast and insect systems. In yeast, a number of vectors containing constitutive or inducible promoters may be used with *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast), *Pichia pastoris*, and *Hansenula polymorpha* (methylotropic yeasts). For a review see, "Current Protocols in Molecular Biology", Vol. 2, 1988, Ausubel *et al.* (eds.), Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant *et al.*, 1987, *Expression and Secretion Vectors for Yeast*, 1987, in "Methods in Enzymology", Wu and Grossman (eds.), Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, *DNA Cloning*, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, *Heterologous Gene Expression in Yeast*, in "Methods in Enzymology", Berger and Kimmel (eds.), Acad. Press, N.Y., Vol. 152, pp. 673-684; and "The Molecular Biology of the Yeast *Saccharomyces*", 1982, Strathern *et al.* (eds.), Cold Spring Harbor Press, Vols. I and II.

In an insect system a baculovirus, *Autographa californica* nuclear polyhidrosis virus (AcNPV), can be used as a vector to express an $\alpha 2M$ polypeptide in *Spodoptera frugiperda* cells. The $\alpha 2M$ polypeptide DNA may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). These recombinant viruses are then used to infect host cells in which the inserted DNA is expressed (see, *e.g.*, Smith *et al.*, 1983, *J. Virol.* 46:584; Smith, U.S. Patent No. 4,215,051).

Any of the cloning and expression vectors described herein may be synthesized and assembled from known DNA sequences by techniques well known in the art. The regulatory regions and enhancer elements can be of a variety of origins, both natural and synthetic. Some vectors and host cells may be obtained commercially. Non-limiting examples of useful vectors are described in Appendix 5 of *Current Protocols in Molecular Biology*, 1988, ed. Ausubel *et al.*, Greene Publish. Assoc. & Wiley Interscience, which is incorporated herein by reference; and the catalogs of commercial suppliers such as Clontech Laboratories, Stratagene Inc., and Invitrogen, Inc.

5.1.1.5 SYNTHETIC PRODUCTION

An alternative to producing $\alpha 2M$ by recombinant techniques is peptide synthesis. For example, a peptide corresponding to a portion of an $\alpha 2M$ comprising the substrate-binding domain, or which binds peptides *in vitro*, can be synthesized by use of a peptide synthesizer. Conventional peptide synthesis may be used or other synthetic protocols well known in the art.

In addition, analogs and derivatives of $\alpha 2M$ polypeptides can be chemically synthesized. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the $\alpha 2M$ sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general.

Peptides having $\alpha 2M$ amino acid sequences, or a fragment, analog, mutant or derivative thereof, may be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, J. Am. Chem. Soc., 85:2149. During synthesis, N- α -protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its C-terminal and to an insoluble polymeric support i.e., polystyrene beads. The peptides are synthesized by linking an amino group of an N- α -deprotected amino acid to an α -carboxyl group of an N- α -protected amino acid that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide. The attachment of a free amino group to the activated carboxyl leads to peptide bond formation. The most commonly used N- α -protecting groups include Boc which is acid labile and Fmoc which is base labile. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art and so are not discussed in detail herein (See, Atherton, *et al.*, 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, and Bodanszky, 1993, Peptide Chemistry, A Practical Textbook, 2nd Ed., Springer-Verlag).

Purification of the resulting $\alpha 2M$ polypeptides accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography. The choice of appropriate matrices and buffers are well known in the art and so are not described in detail herein.

5.2 ANTIGENIC COMPLEXES COMPRISING $\alpha 2M$ POLYPEPTIDES

5.2.1 ISOLATION OF INTRACELLULAR COMPLEXES OF $\alpha 2M$ POLYPEPTIDES WITH ANTIGENIC MOLECULES

Described herein are methods for purifying $\alpha 2M$ polypeptides or $\alpha 2M$ polypeptide-antigenic molecule complexes of the invention from recombinant cells, and with minor modifications known in the art, the $\alpha 2M$ polypeptide or $\alpha 2M$ -antigenic molecule complexes from the cell culture. Recombinant cells include, for example, cells expressing

antigenic molecules and recombinantly expressing an $\alpha 2M$ polypeptide. Such cells may be derived from a variety of sources, including, but not limited to, cells infected with an infectious agent and cancer cells.

5 The invention provides methods for purification of recombinant $\alpha 2M$ polypeptide-antigenic molecule complexes by affinity purification, based on the properties of the affinity label present on the $\alpha 2M$ polypeptide. One approach is based on specific molecular interactions between a tag and its binding partner. The other approach relies on the immunospecific binding of an antibody to an epitope present on the tag. The principle of affinity chromatography well known in the art is generally applicable to both of these approaches.

10 To produce $\alpha 2M$ polypeptide-antigenic molecule complexes, a nucleotide sequence encoding an $\alpha 2M$ polypeptide can be introduced into a cell. When an antigenic molecule is present in the cell, the $\alpha 2M$ polypeptide can associate intracellularly with the antigenic molecule, forming a covalent or a noncovalent complex of $\alpha 2M$ polypeptide and the antigenic molecule. Cells into which an $\alpha 2M$ polypeptide-encoding nucleotide sequence can be introduced, include, but are not limited to, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc. The choice of cell type depends on the type of tumor or infectious disease being treated or prevented, and can be determined by one of skill in the art. In a specific embodiment, an expression construct comprising a nucleic acid sequence encoding the $\alpha 2M$ polypeptide is introduced into an antigenic cell. As used herein, antigenic cells may include cells that are infected with an infectious agent or pathogen, cells infected with non-infectious or non-pathogenic forms of an infectious agent or pathogen (*e.g.*, by use of a helper infectious agent), cells infected by or engineered to express an attenuated form of an infectious agent or a non-pathogenic or replication-deficient variant of a pathogen, pre-neoplastic cells that are infected with a cancer-causing infectious agent, such as a virus, but which are not yet neoplastic; or antigenic cells that have been exposed to a mutagen or cancer-causing agent, such as, for example DNA-damaging agents, radiation, etc. Other cells that can be used are pre-neoplastic cells which are in transition from a normal to a neoplastic form as characterized by morphology, physiological or biochemical functions. Preferably, the cancer cells and pre-neoplastic cells used in the methods of the invention are of mammalian origin. Mammals contemplated by this aspect of the invention include humans, companion animals (*e.g.*, dogs and cats), livestock animals (*e.g.*, sheep, cattle, goats, pigs and horses), laboratory animals (*e.g.*, mice, rats and rabbits), and captive or free wild animals.

In various embodiments, any cancer cell, preferably a human cancer cell, can be used in the present methods for producing α 2M polypeptide-antigenic molecule complexes. The cancer cells provide the antigenic peptides which become associated covalently or noncovalently with the expressed α 2M polypeptide. α 2M polypeptide-antigenic molecule complexes are then purified from the cells and used to treat such cancers. Cancers which can be treated or prevented with immunogenic compositions prepared by methods of the invention include, but are not limited to, tumors such as sarcomas and carcinomas. Examples of cancers that are amenable to the methods of the invention are listed in Section 5.6. Accordingly, any tissues or cells isolated from a pre-neoplastic lesion, a cancer, including cancer that has metastasized to multiple remote sites, can be used in the present method. For example, cells found in abnormally growing tissue, circulating leukemic cells, metastatic lesions as well as solid tumor tissue can be used.

In another embodiment, cell lines derived from a pre-neoplastic lesion, cancer tissues or cancer cells can also be used, provided that the cells of the cell line have at least one or more antigenic determinants in common with antigens on the target cancer cells. Cancer tissues, cancer cells, cells infected with a cancer-causing agent, other pre-neoplastic cells, and cell lines of human origin are preferred.

Cancer and pre-neoplastic cells can be identified by any method known in the art. For example, cancer cells can be identified by morphology, enzyme assays, proliferation assays, cytogenetic characterization, DNA mapping, DNA sequencing, the presence of cancer-causing virus, or a history of exposure to mutagen or cancer-causing agent, imaging, etc. Cancer cells may also be obtained by surgery, endoscopy, or other biopsy techniques. If some distinctive characteristics of the cancer cells are known, they can also be obtained or purified by any biochemical or immunological methods known in the art, such as but not limited to affinity chromatography, and fluorescence activated cell sorting (*e.g.*, with fluorescently tagged antibody against an antigen expressed by the cancer cells).

Cancer tissues, cancer cells or cell lines may be obtained from a single individual or pooled from several individuals. It is not essential that clonal, homogeneous, or purified population of cancer cells be used. It is also not necessary to use cells of the ultimate target *in vivo* (*e.g.*, cells from the tumor of the intended recipient), so long as at least one or more antigenic determinants on the target cancer cells is present on the cells used for expression of the α 2M polypeptide. In addition, cells derived from distant metastases may be used to prepare an immunogenic composition against the primary cancer. A mixture of cells can be used provided that a substantial number of cells in the mixture are cancer cells and share at least one antigenic determinant with the target cancer cell. In a specific embodiment, the cancer cells to be used in expressing an α 2M polypeptide are purified.

5.2.2 *IN VITRO* COMPLEXING

In another embodiment, complexes of $\alpha 2M$ polypeptides and antigenic molecules are produced *in vitro*. Immunogenic $\alpha 2M$ polypeptide – antigenic molecule complexes can be generated *in vitro* by any method known in the art for forming $\alpha 2M$ polypeptide – antigenic molecule complexes. Procedures for forming such $\alpha 2M$ –antigenic molecule complexes and methods for isolating antigenic peptides are described in detail herein.

Methods for formation *in vitro* of noncovalent immunogenic complexes are well known in the art. For example, such complexes can be generated *in vitro* by noncovalent complexing of an $\alpha 2M$ polypeptide with an antigenic molecule using methods which have been previously described for noncovalent coupling of an HSP with an antigenic molecule (see *e.g.*, Blachere *et al.*, 1997, *supra*; PCT publication WO 97/10000, dated March 20, 1997). Preferably, the immunogenic molecular complex is not prepared by treatment with a protease, or with an activating agent such as ammonia or methyamine. In another preferred embodiment, the $\alpha 2M$ molecule of the immunogenic molecular complex is not cleaved within the “bait” region. In yet another embodiment, the $\alpha 2M$ polypeptide is not covalently associated with the antigenic molecule through a thioester linkage.

Methods for covalent coupling are also well known in the art (see, *e.g.*, Osada *et al.*, 1987, *supra*; Osada *et al.*, 1988, *supra*; Chu and Pizzo 1993, *supra*; Chu *et al.*, 1994, *supra*; Mitsuda *et al.*, 1993, *supra*). In one embodiment, for example, when an $\alpha 2M$ polypeptide is mixed with protease,, During proteolytic activation of $\alpha 2M$, non-proteolytic ligands can become covalently bound to the activated thioesters. Non-proteolytic ligands can also be incorporated into the activated $\alpha 2M$ molecule by ammonia or methylamine during reversal of the nucleophilic activation, employing heat (Grøn and Pizzo, 1998, *Biochemistry*, 37: 6009-6014). Such conditions that allow fortuitous trapping of peptides by $\alpha 2M$ are employed to prepare the $\alpha 2M$ polypeptide – antigenic molecule complexes of the invention.

For example, in various embodiments of the invention, an $\alpha 2M$ polypeptide may be mixed with antigenic molecule in the presence of a protease, ammonia or other small amine nucleophiles such as methylamine and ethylamine. Non-limiting examples of proteases which may be used include trypsin, porcine pancreatic elastase (PEP), human neutrophil elastase, cathepsin G, *S. aureus* V-8 proteinase trypsin, a-chymotrypsin, V8 protease, papain, and proteinase K (see Ausubel *et al.*, (eds.), in “Current Protocols in Molecular Biology”, Greene Publishing Associates and Wiley Interscience, New York, 17.4.6-17.4.8).

In another embodiment for preparation of covalent $\alpha 2M$ polypeptide–antigenic molecule complexes, $\alpha 2M$ polypeptides and antigenic molecules are prepared, and then covalently coupled using, for example, chemical crosslinking. Chemical crosslinking methods are well known in the art. For example, in a preferred embodiment, glutaraldehyde crosslinking may be used. Glutaraldehyde crosslinking has been used for formation of

covalent complexes of peptides and hsp (see Barrios *et al.*, 1992, Eur. J. Immunol. 22: 1365-1372). In one embodiment, the following protocol is used. Optionally, α 2M polypeptides may be pretreated with ATP or low pH prior to complexing, in order to remove any peptides that may be associated with the α 2M polypeptide. Preferably, 1 mg of α 2M polypeptide is crosslinked to 1 mg of peptide in the presence of 0.002% glutaraldehyde for 2 hours. Glutaraldehyde is removed by dialysis against phosphate buffered saline (PBS) overnight (Lussow *et al.*, 1991, Eur. J. Immunol. 21: 2297-2302).

Other methods for chemical crosslinking may also be used, in addition other methods for covalent attachment of proteins, such as photocrosslinking (see Current Protocols in Molecular Biology, Ausubel *et al.* (eds.), Greene Publishing Associates and Wiley Interscience, New York).

Antigenic molecules for covalent or noncovalent α 2M polypeptide-antigenic molecule complexes may be isolated from various sources, chemically synthesized, or produced recombinantly. Such methods can be readily adapted for medium or large scale production of the immunotherapeutic or prophylactic vaccines of the invention.

Following complexing, the immunogenic α 2M-antigenic molecule complexes can optionally be purified. In a preferred embodiment, such complexes are at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100% noncovalent complexes of α 2M and the antigenic molecule. Such complexes may be assayed *in vitro* using, for example, the mixed lymphocyte target cell assay (MLTC) described below. Once immunogenic complexes have been isolated they can be optionally characterized further in animal models using the preferred administration protocols and excipients discussed below.

5.2.3. α 2M – ANTIGENIC MOLECULE FUSION PROTEINS

In another embodiment, recombinant fusion proteins, comprised of α 2M sequences linked to antigenic molecule sequences, may be used for immunotherapy. To produce such a recombinant fusion protein, an expression vector is constructed using nucleic acid sequences encoding α 2M fused to sequences encoding an antigenic molecule, using recombinant methods known in the art, such as those described in Sections 5.1.1.1 and 5.1.1.2, above (see Suzue *et al.*, 1997, Proc. Natl. Acad. Sci. U.S.A. 94: 13146-51). α 2M-antigenic peptide fusions are then expressed and isolated. By specifically designing the antigenic peptide portion of the molecule, such fusion proteins can be used to elicit an immune response and in immunotherapy against target cancer and infectious diseases.

5.2.4 SOURCES OF ANTIGENIC MOLECULES

Antigenic molecules, or antigenic portions thereof, specific to one or more types of cancer or infected cells, can be chosen from among those known in the art. Alternatively, such antigenic molecules can be selected for their antigenicity or their immunogenicity, as determined by immunoassays or by their ability to generate an immune response.

5.2.4.1 EXOGENOUS ANTIGENIC MOLECULES

Preferably, where it is desired to treat or prevent cancer, known tumor-specific antigenic molecules or fragments or derivatives thereof are used. For example, such tumor specific or tumor-associated antigenic molecules include but are not limited to KS 1/4 pan-carcinoma antigen (Perez and Walker, 1990, J. Immunol. 142:3662-3667; Bumal, 1988, Hybridoma 7(4):407-415); ovarian carcinoma antigen (CA125) (Yu, *et al.*, 1991, Cancer Res. 51(2):468-475); prostatic acid phosphate (Tailer, *et al.*, 1990, Nucl. Acids Res. 18(16):4928); prostate specific antigen (Henttu and Vihko, 1989, Biochem. Biophys. Res. Comm. 160(2):903-910; Israeli, *et al.*, 1993, Cancer Res. 53:227-230); melanoma-associated antigen p97 (Estin, *et al.*, 1989, J. Natl. Cancer Inst. 81(6):445-446); melanoma antigen gp75 (Vijayasardahl, *et al.*, 1990, J. Exp. Med. 171(4):1375-1380); high molecular weight melanoma antigen (Natali, *et al.*, 1987, Cancer 59:55-63) and prostate specific membrane antigen.

In a specific embodiment, an antigenic molecule or fragment or derivative thereof specific to a certain tumor is selected for complexing to α 2M polypeptide and subsequent administration to a patient having that tumor.

In a preferred embodiment, where it is desired to treat or prevent viral diseases, molecules comprising epitopes of known viruses are used. For example, such antigenic epitopes may be prepared from viruses including, but not limited to, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I), and human immunodeficiency virus type II (HIV-II).

In another preferred embodiment, where it is desired to treat or prevent bacterial infections, molecules comprising epitopes of known bacteria are used. For example, such antigenic epitopes may be prepared from bacteria including, but not limited to, mycobacteria rickettsia, mycoplasma, neisseria and legionella.

In another preferred embodiment, where it is desired to treat or prevent protozoal infections, molecules comprising epitopes of known protozoa are used. For example, such

antigenic epitopes may be prepared from protozoa including, but not limited to, leishmania, kokzidioa, and trypanosoma.

In yet another preferred embodiment, where it is desired to treat or prevent parasitic infections, molecules comprising epitopes of known parasites are used. For example, such antigenic epitopes may be from parasites including, but not limited to, chlamydia and rickettsia.

To determine immunogenicity or antigenicity of a putative antigen by detecting binding to antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in vivo* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, immunoprecipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one aspect, antibody binding is detected by detecting a label on the primary antibody. In another aspect, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further aspect, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are envisioned for use. In one embodiment for detecting immunogenicity, T cell-mediated responses can be assayed by standard methods, *e.g.*, *in vitro* cytotoxicity assays or *in vivo* delayed-type hypersensitivity assays.

Potentially useful antigenic molecules, or derivatives thereof, can be identified by various criteria, such as the antigen's involvement in neutralization of a pathogen's infectivity (wherein it is desired to treat or prevent infection by such a pathogen) (Norrby, 1985, *Summary*, in Vaccines 85, Lerner, *et al.* (eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 388-389), type or group specificity, recognition by patients' antisera or immune cells, and/or the demonstration of protective effects of antisera or immune cells specific for the antigen. In addition, where it is desired to treat or prevent a disease caused by pathogen, the antigen's encoded epitope should preferably display a small or no degree of antigenic variation in time or amongst different isolates of the same pathogen.

5.2.4.2 ANTIGENIC MOLECULES FROM α 2M COMPLEXES

Antigenic peptides for complexing *in vitro* to α 2M polypeptides of the invention can also be obtained from endogenous complexes of peptides and α 2Ms. Two methods may be used to elute the peptide from an α 2M-antigenic molecule complex. One approach involves

incubating the $\alpha 2$ M-antigenic molecule complex in the presence of ATP. The other approach involves incubating the complexes in a low pH buffer.

5 Briefly, the complex of interest is centrifuged through a Centricon 10 assembly (Millipore) to remove any low molecular weight material loosely associated with the complex. The large molecular weight fraction may be removed and analyzed by SDS-PAGE while the low molecular weight may be analyzed by HPLC as described below. In the ATP incubation protocol, the $\alpha 2$ M-antigenic molecule complex in the large molecular weight fraction is incubated with 10mM ATP for 30 minutes at room temperature. In the low pH protocol, acetic acid or trifluoroacetic acid (TFA) is added to the $\alpha 2$ M-antigenic molecule complex to give a final concentration of 10% (vol/vol) and the mixture incubated at room temperature or in a boiling water bath or any temperature in between, for 10 minutes.

10 The resulting samples are centrifuged through a Centricon 10 assembly as mentioned previously. The high and low molecular weight fractions are recovered. The remaining large molecular weight $\alpha 2$ M-antigenic molecule complexes can be reincubated with ATP or low pH to remove any remaining peptides.

15 The resulting lower molecular weight fractions are pooled, concentrated by evaporation and dissolved in 0.1% TFA. The dissolved material is then fractionated by reverse phase high pressure liquid chromatography (HPLC) using for example a VYDAC C18 reverse phase column equilibrated with 0.1% TFA. The bound material is then eluted at a flow rate of about 0.8 ml/min by developing the column with a linear gradient of 0 to 80% acetonitrile in 0.1% TFA. The elution of the peptides can be monitored by OD₂₁₀ and the fractions containing the peptides collected.

5.2.4.3 PEPTIDE ANTIGENS FROM MHC COMPLEXES

25 Peptides bound to MHC molecules *in vivo* can also be used *in vitro* to form complexes with $\alpha 2$ M polypeptides of the invention. The isolation of potentially immunogenic peptides from MHC molecules is well known in the art and so is not described in detail herein (*see*, Falk, *et al.*, 1990, Nature 348:248-251; Rotzsche, *et al.*, 1990, Nature 348:252-254; Elliott, *et al.*, 1990, Nature 348:191-197; Falk, *et al.*, 1991, Nature 351:290-296; Demotz, *et al.*, 1989, Nature 343:682-684; Rotzsche, *et al.*, 1990, Science 249:283-287), the disclosures of which are incorporated herein by reference.

30 Briefly, MHC-antigenic molecule complexes may be isolated by a conventional immunoaffinity procedure. The peptides then may be eluted from the MHC-antigenic molecule complex by incubating the complexes in the presence of about 0.1% TFA in acetonitrile. The eluted peptides may be fractionated and purified by reverse phase HPLC, as before.

5.2.4.4 SYNTHETIC ANTIGENIC MOLECULES

The amino acid sequences of the peptides eluted from MHC molecules or $\alpha 2M$ may be determined either by manual or automated amino acid sequencing techniques well known in the art. Once the amino acid sequence of a potentially protective peptide has been determined, the peptide may be synthesized in using conventional peptide synthesis or other protocols well known in the art.

Peptides having the same amino acid sequence as those isolated above may be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, J. Am. Chem. Soc., 85:2149. During synthesis, N- α -protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its C-terminal and to an insoluble polymeric support i.e., polystyrene beads. The peptides are synthesized by linking an amino group of an N- α -deprotected amino acid to an α -carboxyl group of an N- α -protected amino acid that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide. The attachment of a free amino group to the activated carboxyl leads to peptide bond formation. The most commonly used N- α -protecting groups include Boc which is acid labile and Fmoc which is base labile. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art and so are not discussed in detail herein (*See*, Atherton, *et al.*, 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, and Bodanszky, 1993, Peptide Chemistry, A Practical Textbook, 2nd Ed., Springer-Verlag).

Purification of the resulting peptides is accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography. The choice of appropriate matrices and buffers are well known in the art and so are not described in detail herein.

5.2.4.5 RECOMBINANTLY PRODUCED ANTIGENIC MOLECULES

In a particular embodiment of the invention, a nucleotide sequence encoding a protein antigenic molecule or portions thereof can be introduced into a host cell for production of the antigenic molecule. The nucleotide sequence encoding any antigenic protein can be obtained and cloned into an expression vector for expression essentially by the same methods described for the cloning and expression of a nucleotide sequence encoding an $\alpha 2M$ polypeptide. The techniques are described in Sections 5.1.1.1 and 5.1.1.2, and are well known in the art. The recombinant antigenic protein or portions thereof can be purified by any methods appropriate for the protein, and then used to form complexes with $\alpha 2M$ polypeptides *in vitro* as described in Section 5.2.2. Such an $\alpha 2M$ polypeptide-antigenic molecule complex can be used as a vaccine to stimulate an immune response against the

antigenic protein in a subject for the purpose of treatment or prevention of infectious diseases or cancer.

5.3 THERAPEUTIC APPLICATIONS FOR α 2M COMPLEXES

5 The present invention encompasses the use of α 2M polypeptides in methods for treatment of and prevention of infectious diseases and cancer. In various embodiments described in detail herein, an effective amount of a α 2M polypeptide in a covalent or noncovalent complex with an antigenic molecule is administered to a patient for therapeutic purposes.

10

5.3.1 PREVENTION AND TREATMENT OF INFECTIOUS DISEASES

For treatment and prevention of infectious disease, α 2M – antigenic molecule complexes are prepared from a cell that displays the antigenicity of an antigen of an infectious agent or pathogenic agent, and used as vaccines against the infectious disease. As
15 will be appreciated by those skilled in the art, the protocols described herein may be used to isolate α 2M polypeptide–antigenic molecule complexes from any cell that displays the antigenicity of an antigen of the infectious agent. For example, cells may be infected by the infectious agent itself, or alternatively, cells may be infected by or engineered to express an attenuated form of the infectious agent or a non-pathogenic or replication-deficient variant of
20 the pathogen. In one embodiment, α 2M– antigenic molecule complexes can be prepared from cells infected with non-infectious or non-pathogenic forms of the infectious agent (*e.g.*, by use of a helper infectious agent). In another embodiment, the α 2M–antigenic molecule complexes of the invention may be prepared from cells infected with an intracellular pathogen. In another embodiment, α 2M polypeptide-complexes can be prepared from cells
25 that have been transformed by an intracellular pathogen. For example, immunogenic α 2M polypeptide–antigenic molecule complexes may be isolated from eukaryotic cells transformed with a transforming virus such as SV40.

A preferred method for treatment or prevention of an infectious disease comprises introducing into a cell that displays the antigenicity of an infectious agent an expressible
30 α 2M polypeptide gene sequence, preferably as an expression gene construct. The α 2M polypeptide gene sequence is manipulated by recombinant methods, such as those described above in Sections 5.1.1.1 and 5.1.1.2 above, so that the α 2M polypeptide gene sequence, in the form of an expression construct, located extrachromosomally or integrated in the chromosome, is suitable for expression of the α 2M polypeptide in the recombinant cells.
35 The recombinant cells containing the expression gene constructs are cultured under conditions such that α 2M polypeptides encoded by the expression gene construct are

expressed. Complexes of $\alpha 2M$ polypeptides covalently or noncovalently associated with antigenic molecules of the infectious agent are purified from the cell culture or culture medium by the methods described in Section 5.2.1.

5 In various embodiments, $\alpha 2M$ – antigenic molecule complexes are prepared from a cell genetically manipulated to express an $\alpha 2M$ polypeptide, for example, tissues, isolated
10 cells or immortalized eukaryotic cell lines infected with an intracellular pathogen. When immortalized animal cell lines are used as a source of the $\alpha 2M$ polypeptide–antigenic molecule complex, it is important to use cell lines that can be infected with the pathogen of interest. In addition, it is preferable to use cells that are derived from the same species as the
15 intended recipient of the vaccine. Techniques for introducing an expressible form of the $\alpha 2M$ polypeptide gene sequences into these cell lines are described above in Section 5.1.1.2. If a pathogen is expected to cause lysis of the host cells, it is preferred to introduce the expressible $\alpha 2M$ polypeptide gene sequence into the host cell prior to infecting the cells with the pathogen. For example, in order to prepare an $\alpha 2M$ polypeptide–antigenic molecule
20 complex for administration to humans that may be effective against HIV-1, the virus may be propagated in human cells which include, but are not limited to, human CD4+ T cells, HepG2 cells, and U937 promonocytic cells, which have already been transfected with an expressible $\alpha 2M$ polypeptide sequence. Similarly, influenza viruses may be propagated in, for example, transfected human fibroblast cell lines and MDCK cells, and mycobacteria may be cultured in, for example, transfected human Schwann cells. The cell supernatant
25 containing $\alpha 2M$ –antigenic molecule complex may be collected just prior to lysis of the host cell.

In a preferred aspect of the invention, the purified $\alpha 2M$ – antigenic molecule complex vaccines may have particular utility in the treatment of human diseases caused by
30 intracellular pathogens. It is appreciated, however, that the vaccines developed using the principles described herein will be useful in treating diseases of other mammals, for example, farm animals including: cattle; horses; sheep; goats; and pigs, and household pets including: cats; and dogs, that similarly are caused by intracellular pathogens.

In accordance with the methods described herein, vaccines may be prepared that
35 stimulate an immune response, in particular a cytotoxic T cell responses, against cells infected with viruses including, but not limited to, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, HSV-I, HSV-II, rinderpest rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsachie virus, mumps virus, measles virus, rubella virus, polio virus, HIV-I, and HIV-II. Similarly, vaccines may also be prepared that
40 stimulate cytotoxic T cell responses against cells infected with intracellular bacteria, including, but not limited to, *Mycobacteria*, *Rickettsia*, *Mycoplasma*, *Neisseria* and

Legionella. In addition, vaccines may also be prepared that stimulate cytotoxic T cell responses against cells infected with intracellular protozoa, including, but not limited to, *Leishmani*, *Kokzidioa*, and *Trypanosoma*. Furthermore, vaccines may be prepared that stimulate cytotoxic T cell responses against cells infected with intracellular parasites including, but not limited to, *Chlamydia* and *Rickettsia*.

The effect of immunotherapy with modified $\alpha 2M$ polypeptide-antigenic molecule complexes on progression of infectious diseases can be monitored by any methods known to one skilled in the art.

5.3.2 PREVENTION AND TREATMENT OF CANCER

There are many reasons why immunotherapy as provided by the covalent or noncovalent $\alpha 2M$ polypeptide-antigenic molecule complexes or recombinant cells expressing $\alpha 2M$ polypeptides prepared by the present invention is desired for use in cancer patients. First, if cancer patients are immunosuppressed, and surgery with anesthesia, and subsequent chemotherapy, may worsen the immunosuppression, then with appropriate immunotherapy in the preoperative period, this immunosuppression may be prevented or reversed. This could lead to fewer infectious complications and to accelerated wound healing. Second, tumor bulk is minimal following surgery and immunotherapy is most likely to be effective in this situation. A third reason is the possibility that tumor cells are shed into the circulation at surgery and effective immunotherapy applied at this time can eliminate these cells.

In a specific embodiment, the preventive and therapeutic utility of the invention is directed at enhancing the immunocompetence of the cancer patient either before surgery, at or after surgery, and at inducing tumor-specific immunity to cancer cells, with the objective being inhibition of cancer, and with the ultimate clinical objective being total cancer regression and eradication.

According to the invention, preferred methods of treatment or prevention of cancer comprise isolating cancer cells from one or more individual, preferably the individual in need of treatment, and introducing into such cells an expressible $\alpha 2M$ polypeptide gene sequence, preferably as an expression gene construct. The $\alpha 2M$ polypeptide gene sequence is manipulated by methods described above in Sections 5.1.1.1 and 5.1.1.2, such that the $\alpha 2M$ polypeptide gene sequence, in the form of an expression construct, or intrachromosomally integrated, are suitable for expression of the $\alpha 2M$ polypeptide in the recombinant cells. The recombinant cells containing the expression gene constructs are cultured under conditions such that $\alpha 2M$ polypeptides encoded by the expression gene construct are expressed by the recombinant host cells. Complexes of $\alpha 2M$ polypeptides covalently or noncovalently associated with antigenic molecules of the cancer cell are purified from the cell culture or culture medium by the methods described in Section 5.2.1. Depending on the route of

administration, the $\alpha 2M$ polypeptide-antigenic molecule complexes are formulated accordingly as described in Section 5.7, and administered to the individual autologously (e.g., to treat the primary cancer or metastases thereof), or to other individuals who are in need of treatment for cancer of a similar tissue type, or to individuals at enhanced risk of cancer due to familial history or environmental risk factors.

5 For example, treatment with $\alpha 2M$ polypeptide – antigenic molecule complexes prepared as described above may be started any time after surgery. However, if the patient has received chemotherapy, $\alpha 2M$ – antigenic molecule complexes are usually administered after an interval of four weeks or more so as to allow the immune system to recover. The therapeutic regimen may include weekly injections of the $\alpha 2M$ polypeptide – antigenic molecule complex, dissolved in saline or other physiologically compatible solution. The route and site of injection is varied each time, for example, the first injection is given subcutaneously on the left arm, the second injection on the right arm, the third injection on the left abdominal region, the fourth injection on the right abdominal region, the fifth injection on the left thigh, the sixth injection on the right thigh, *etc.* The same site is repeated after a gap of one or more injections. In addition, injections are split and each half of the dose is administered at a different site on the same day. Overall, the first four to six injections are given at weekly intervals. Subsequently, two injections are given at two-week intervals, followed by a regimen of injections at monthly intervals.

20 Alternatively, recombinant tumor cells expressing $\alpha 2M$ – antigenic molecule complexes can be used as a vaccine for injection into a patient to stimulate an immune response against the tumor cells or cells bearing tumor antigens. Autologous recombinant tumor cells stably expressing $\alpha 2M$ polypeptide-antigenic molecule complexes are preferred. To determine the appropriate dose, the amount of $\alpha 2M$ polypeptide-antigenic molecule complex produced by the recombinant cells is quantitated, and the number of recombinant cells used for vaccination is adjusted accordingly to assure a consistent level of expression *in vivo*. A preferred dose is the number of recombinant cells that can produce about 100 ng $\alpha 2M$ polypeptide per 24 hours. For the safety of the patient, the recombinant tumor cells can be irradiated (12000 rad) immediately prior to injection into a patient. Irradiated cells do not proliferate, and can continue to express $\alpha 2M$ polypeptide-antigenic molecule complexes for about 7-10 days which is sufficient to induce an immune response.

30 Cancers that can be treated or prevented by using covalent or noncovalent $\alpha 2M$ -antigenic molecule complexes prepared by the methods of the present invention include, but not limited to human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma,

pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular
5 tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and
10 erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

15 In a specific embodiment, the cancer is metastatic. In another specific embodiment, the patient having a cancer is immunosuppressed by reason of having undergone anti-cancer therapy (*e.g.*, chemotherapy radiation) prior to administration of the $\alpha 2M$ - antigenic molecule complexes of the invention. In another specific embodiment, the cancer is a tumor.

The effect of immunotherapy with $\alpha 2M$ polypeptide-antigenic molecule complexes
20 on progression of neoplastic diseases can be monitored by any methods known to one skilled in the art, including but not limited to measuring: a) delayed hypersensitivity as an assessment of cellular immunity; b) activity of cytolytic T-lymphocytes *in vitro*; c) levels of tumor specific antigens, *e.g.*, carcinoembryonic (CEA) antigens; d) changes in the morphology of tumors using techniques such as a computed tomographic (CT) scan; e)
25 changes in levels of putative biomarkers of risk for a particular cancer in individuals at high risk, and f) changes in the morphology of tumors using a sonogram. Other techniques that can also be used include scintigraphy and endoscopy.

The preventive effect of immunotherapy using $\alpha 2M$ polypeptide-antigenic molecule complexes may also be estimated by determining levels of a putative biomarker for risk of a
30 specific cancer. For example, in individuals at enhanced risk for prostate cancer, serum prostate-specific antigen (PSA) is measured by the procedure described by Brawer *et al.*, 1992, *J. Urol.* 147:841-845, and Catalona *et al.*, 1993, *JAMA* 270:948-958; or in individuals at risk for colorectal cancer, CEA is measured by methods known in the art; and in
individuals at enhanced risk for breast cancer, 16- α -hydroxylation of estradiol is measured by
35 the procedure described by Schneider *et al.*, 1982, *Proc. Natl. Acad. Sci. USA* 79:3047-3051. The references cited above are incorporated by reference herein in their entirety.

5.3.3 COMBINATION WITH ADOPTIVE IMMUNOTHERAPY

Adoptive immunotherapy refers to a therapeutic approach for treating infectious diseases or cancer in which immune cells are administered to a host with the aim that the cells mediate specific immunity, either directly or indirectly, to the infected cells or tumor cells and/or antigenic components, and result in treatment of the infectious disease or regression of the tumor, as the case may be (see U.S. Patent Application Serial No. 08/527,546, filed September 13, 1995, which is incorporated by reference herein in its entirety). α 2M polypeptides may be used to sensitize antigen presenting cells (APCs) using in covalent or noncovalent complexes with antigenic (or immunogenic) molecules, for adoptive immunotherapy.

According to the invention, therapy by administration of α 2M polypeptide-antigenic molecule complexes, using any desired route of administration, is combined with adoptive immunotherapy using APC sensitized with α 2M polypeptide-antigenic molecule complexes. The α 2M polypeptide-antigenic molecule complex-sensitized APC can be administered concurrently with α 2M polypeptide-antigenic molecule complexes, or before or after administration of α 2M polypeptide-antigenic molecule complexes. Furthermore, the mode of administration can be varied, including but not limited to, *e.g.*, subcutaneously, intravenously, intraperitoneally, intramuscularly, intradermally or mucosally.

5.3.3.1 SENSITIZATION OF ANTIGEN PRESENTING CELLS WITH α 2M COMPLEXES

The antigen-presenting cells, including but not limited to macrophages, dendritic cells and B-cells, are preferably obtained by production *in vitro* from stem and progenitor cells from human peripheral blood or bone marrow as described by Inaba *et al.*, 1992, J. Exp. Med. 176:1693-1702. APC can be obtained by any of various methods known in the art. In a preferred aspect human macrophages are used, obtained from human blood cells.

By way of example, but not limitation, macrophages can be obtained as follows: Mononuclear cells are isolated from peripheral blood of a patient (preferably the patient to be treated), by Ficoll-Hypaque gradient centrifugation and are seeded on tissue culture dishes which are pre-coated with the patient's own serum or with other AB+ human serum. The cells are incubated at 37°C for 1 hr, then non-adherent cells are removed by pipetting. To the adherent cells left in the dish, is added cold (4°C) 1 mM EDTA in phosphate-buffered saline and the dishes are left at room temperature for 15 minutes. The cells are harvested, washed with RPMI buffer and suspended in RPMI buffer. Increased numbers of macrophages may be obtained by incubating at 37°C with macrophage-colony stimulating factor (M-CSF); increased numbers of dendritic cells may be obtained by incubating with

granulocyte-macrophage-colony stimulating factor (GM-CSF) as described in detail by Inaba, K., *et al.*, 1992, J. Exp. Med. 176:1693-1702.

APC are sensitized with $\alpha 2M$ polypeptides covalently or noncovalently bound to antigenic molecules by incubating the cells *in vitro* with the complexes. The APC are sensitized with complexes of $\alpha 2M$ polypeptide and antigenic molecules preferably by incubating *in vitro* with the $\alpha 2M$ polypeptide-complex at 37°C for 15 minutes to 24 hours. By way of example but not limitation, 4×10^7 macrophages can be incubated with 10 microgram $\alpha 2M$ -antigenic molecule complexes per ml or 100 microgram $\alpha 2M$ -antigenic molecule complexes per ml at 37°C for 15 mins to 24 hrs in 1 ml plain RPMI medium. The cells are washed three times and resuspended in a physiological medium preferably sterile, at a convenient concentration (*e.g.*, 1×10^7 /ml) for injection in a patient. In a preferred embodiment, the antigen presenting cells are autologous to the patient, that is, the patient into which the sensitized APCs are injected is the patient from which the APC were originally isolated.

Optionally, the ability of sensitized APC to stimulate, for example, the antigen-specific, class I-restricted cytotoxic T-lymphocytes (CTL) can be monitored by their ability to stimulate CTLs to release tumor necrosis factor, and by their ability to act as targets of such CTLs.

5.3.3.2 REINFUSION OF SENSITIZED APC

The $\alpha 2M$ polypeptide-antigen-sensitized APC are reinfused into the patient systemically, preferably intravenously, by conventional clinical procedures. These activated cells are reinfused, preferentially by systemic administration into the autologous patient. Patients generally receive from about 10^6 to about 10^{12} sensitized macrophages, depending on the condition of the patient. In some regimens, patients may optionally receive in addition a suitable dosage of a biological response modifier including but not limited to the cytokines IFN- α , IFN- γ , IL-2, IL-4, IL-6, TNF or other cytokine growth factor.

5.3.4 DETERMINATION OF IMMUNOGENICITY OF $\alpha 2M$ -ANTIGEN MOLECULE COMPLEXES

In an optional procedure, the purified $\alpha 2M$ polypeptide-antigenic molecule complexes can be assayed for immunogenicity using the mixed lymphocyte target culture assay (MLTC) well known in the art.

By way of example but not limitation, the following procedure can be used. Briefly, mice are injected subcutaneously with the candidate $\alpha 2M$ polypeptide-antigenic molecule complexes. As a positive control another set of mice are immunized with whole cancer

cells of the type from which the $\alpha 2M$ polypeptides are derived. As a negative control, mice are injected with either $\alpha 2M$ - antigenic molecule complexes isolated from normal, non-recombinant cells or whole cells (*i.e.*, antigenically distinct from the type of cell from which the $\alpha 2M$ polypeptides are derived). The mice are injected twice, 7-10 days apart. The mice are injected twice, 7-10 days apart. Ten days after the last immunization, the spleens are removed and the lymphocytes released. The released lymphocytes may be restimulated subsequently in vitro by the addition of dead cells that expressed the complex of interest.

For example, 8×10^6 immune spleen cells may be stimulated with 4×10^4 mitomycin C treated or γ -irradiated (5-10,000 rads) pathogen-infected cells (or cells transfected with a gene encoding an antigen of the infectious agent, as the case may be), or tumor cells in 3ml RPMI medium containing 10% fetal calf serum. In certain cases 33% secondary mixed lymphocyte culture supernatant or interleukin 2 (IL-2) may be included in the culture medium as a source of T cell growth factors (See, Glasebrook *et al.*, 1980, J. Exp. Med. 151:876). To test the primary cytotoxic T cell response after immunization, spleen cells may be cultured without stimulation. In some experiments spleen cells of the immunized mice may also be restimulated with antigenically distinct cells, to determine the specificity of the cytotoxic T cell response.

Six days later the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr -release assay (See, Palladino *et al.*, 1987, Cancer Res. 47:5074-5079 and Blachere, *et al.*, 1993, J. Immunotherapy 14:352-356). In this assay, the mixed lymphocyte culture is added to a target cell suspension to give different effector:target (E:T) ratios (usually 1:1 to 40:1). The target cells are prelabelled by incubating 1×10^6 target cells in culture medium containing 200 mCi ^{51}Cr /ml for one hour at 37°C . The cells are washed three times following labeling. Each assay point (E:T ratio) is performed in triplicate and the appropriate controls incorporated to measure spontaneous ^{51}Cr release (no lymphocytes added to assay) and 100% release (cells lysed with detergent). After incubating the cell mixtures for 4 hours, the cells are pelleted by centrifugation at 200g for 5 minutes. The amount of ^{51}Cr released into the supernatant is measured by a gamma counter. The percent cytotoxicity is measured as cpm in the test sample minus spontaneously released cpm divided by the total detergent released cpm minus spontaneously released cpm.

In order to block the MHC class I cascade a concentrated hybridoma supernatant derived from K-44 hybridoma cells (an anti-MHC class I hybridoma) is added to the test samples to a final concentration of 12.5% (Heike *et al.*, 1994, J. Immunotherapy 15:165-174).

An alternative to the chromium-release assay is the ELISPOT assay which measures cytokine release by cytotoxic T cells in vitro after stimulation with specific antigen. Cytokine release is detected by antibodies which are specific for a particular cytokine, such

as interleukin-2, tumor necrosis factor α or interferon- γ (for example, see Scheibenbogen *et al.*, 1997, *Int. J. Cancer*, 71:932-936). The assay is carried out in a microtiter plate which has been pre-coated with an antibody specific for a cytokine of interest which captures the cytokine secreted by T cells. After incubation of T cells for 24-48 hours in the coated wells, the cytotoxic T cells are removed and replaced with a second labeled antibody that recognizes a different epitope on the cytokine. After extensive washing to remove unbound antibody, an enzyme substrate which produces a colored reaction product is added to the plate. The number of cytokine-producing cells is counted under a microscope. This method has the advantages of short assay time, and sensitivity without the need of a large number of cytotoxic T cells.

5.3.5 MONITORING OF EFFECTS DURING IMMUNOTHERAPY

The effect of immunotherapy with α 2M polypeptide-antigenic molecule complexes can be monitored by any methods known to one skilled in the art, including but not limited to measuring: a) delayed hypersensitivity as an assessment of cellular immunity; b) activity of cytolytic T-lymphocytes *in vitro*; c) levels of infective agent-agent or tumor-specific antigens, *e.g.*, carcinoembryonic (CEA) antigens. In the case of the use of α 2M – antigenic molecule complexes for prevention or treatment of cancer, the effect can additionally be monitored by: d) changes in the morphology of tumors using techniques such as a computed tomographic (CT) scan; and e) changes in levels of putative biomarkers of risk for a particular cancer in individuals at high risk, and f) changes in the morphology of tumors using a sonogram.

5.3.5.1 DELAYED HYPERSENSITIVITY SKIN TEST

Delayed hypersensitivity skin tests are of great value in the overall immunocompetence and cellular immunity to an antigen. Inability to react to a battery of common skin antigens is termed anergy (Sato *et al.*, 1995, *Clin. Immunol. Pathol.* 74:35-43).

Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shortly before use. A 25- or 27-gauge needle ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

5.3.5.2 IN VITRO ACTIVATION OF CYTOTOXIC T CELLS

The activity of cytotoxic T-lymphocytes can be assessed *in vitro* using the following method. Eight x 10⁶ peripheral blood-derived T lymphocytes isolated by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with 4x10⁴ mitomycinC-treated tumor cells in 3ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2, is included in the culture medium as a source of T cell growth factors.

In order to measure the primary response of cytolytic T-lymphocytes after immunization, T cells are cultured without the stimulator tumor cells. In other experiments, T cells are restimulated with antigenically distinct cells. After six days, the cultures are tested for cytotoxicity in a 4 hour ⁵¹Cr-release assay. The spontaneous ⁵¹Cr-release of the targets should reach a level less than 20%. For the anti-MHC class I blocking activity, a tenfold concentrated supernatant of W6/32 hybridoma is added to the test at a final concentration of 12.5% (Heike M., *et al.*, *J. Immunotherapy* 15:165-174).

5.3.5.3 LEVELS OF TUMOR SPECIFIC ANTIGENS

Although it may not be possible to detect unique tumor antigens on all tumors, many tumors display antigens that distinguish them from normal cells. The monoclonal antibody reagents have permitted the isolation and biochemical characterization of the antigens and have been invaluable diagnostically for distinction of transformed from nontransformed cells and for definition of the cell lineage of transformed cells. The best-characterized human tumor-associated antigens are the oncofetal antigens. These antigens are expressed during embryogenesis, but are absent or very difficult to detect in normal adult tissue. The prototype antigen is carcinoembryonic antigen (CEA), a glycoprotein found on fetal gut and human colon cancer cells, but not on normal adult colon cells. Since CEA is shed from colon carcinoma cells and found in the serum, it was originally thought that the presence of this antigen in the serum could be used to screen patients for colon cancer. However, patients with other tumors, such as pancreatic and breast cancer, also have elevated serum levels of CEA. Therefore, monitoring the fall and rise of CEA levels in cancer patients undergoing therapy has proven useful for predicting tumor progression and responses to treatment.

Several other oncofetal antigens have been useful for diagnosing and monitoring human tumors, *e.g.*, alpha-fetoprotein, an alpha-globulin normally secreted by fetal liver and yolk sac cells, is found in the serum of patients with liver and germinal cell tumors and can be used as a marker of disease status.

5.3.5.4 COMPUTED TOMOGRAPHIC (CT) SCAN

CT remains the choice of techniques for the accurate staging of cancers. CT has proved more sensitive and specific than any other imaging techniques for the detection of metastases. A sonogram remains an alternative choice of technique for the accurate staging of cancers.

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5.3.5.5 MEASUREMENT OF PUTATIVE BIOMARKERS

The levels of a putative biomarker for risk of a specific cancer are measured to monitor the effect of $\alpha 2M$ covalently or noncovalently bound to antigenic molecule complexes. For example, in individuals at enhanced risk for prostate cancer, serum prostate-specific antigen (PSA) is measured by the procedure described by Brawer *et. al.*, 1992, J. Urol. 147:841-845, and Catalona *et al.*, 1993, JAMA 270:948-958; and in individuals at enhanced risk for breast cancer, 16- α -hydroxylation of estradiol is measured by the procedure described by Schneider *et al.*, 1982, Proc. Natl. Acad. Sci. USA 79:3047-3051.

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5.4 TARGET AUTOIMMUNE DISEASES

Autoimmune diseases that can be treated by the methods of the present invention include, but are not limited to, insulin dependent diabetes mellitus (*i.e.*, IDDM, or autoimmune diabetes), multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous pemphigoid, discoid lupus, ulcerative colitis, and dense deposit disease. The diseases set forth above, as referred to herein, include those exhibited by animal models for such diseases, such as, for example non-obese diabetic (NOD) mice for IDDM and experimental autoimmune encephalomyelitis (EAE) mice for multiple sclerosis.

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The methods of the present invention can be used to treat such autoimmune diseases by reducing or eliminating the immune response to the patient's own (self) tissue, or, alternatively, by reducing or eliminating a pre-existing autoimmune response directed at tissues or organs transplanted to replace self tissues or organs damaged by the autoimmune response.

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5.5 TARGET INFECTIOUS DISEASES

The infectious diseases that can be treated or prevented using the methods and compositions of the present invention include those caused by intracellular pathogens such as viruses, bacteria, protozoans, and intracellular parasites. Viruses include, but are not limited to viral diseases such as those caused by hepatitis type B virus, parvoviruses, such as adeno-associated virus and cytomegalovirus, papovaviruses such as papilloma virus, polyoma viruses, and SV40, adenoviruses, herpes viruses such as herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), and Epstein-Barr virus, poxviruses, such as variola (smallpox) and vaccinia virus, RNA viruses, including but not limited to human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), human T-cell lymphotropic virus type I (HTLV-I), and human T-cell lymphotropic virus type II (HTLV-II); influenza virus, measles virus, rabies virus, Sendai virus, picornaviruses such as poliomyelitis virus, coxsackieviruses, rhinoviruses, reoviruses, togaviruses such as rubella virus (German measles) and Semliki forest virus, arboviruses, and hepatitis type A virus.

In another embodiment, bacterial infections can be treated or prevented such as, but not limited to disorders caused by pathogenic bacteria including, but not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Salmonella typhi*, *Treponema pallidum*, *Treponema pertenuis*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma spp.*, *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia spp.*, and *Helicobacter pylori*.

In another preferred embodiment, the methods can be used to treat or prevent infections caused by pathogenic protozoans such as, but not limited to, *Entamoeba histolytica*, *Trichomonas tenax*, *Trichomonas hominis*, *Trichomonas vaginalis*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmodium vivax*, *Plasmodium falciparum*, and *Plasmodium malariae*.

5.6 TARGET PROLIFERATIVE CELL DISORDERS

With respect to specific proliferative and oncogenic disease associated with $\alpha 2M$ -
5 $\alpha 2M$ activity, the diseases that can be treated or prevented by the methods of the present invention include, but are not limited to: human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon
10 carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular
15 tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and
20 erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

Diseases and disorders involving a deficiency in cell proliferation or in which cell
25 proliferation is desired for treatment or prevention, and that can be treated or prevented by inhibiting the $\alpha 2M$ function, include but are not limited to degenerative disorders, growth deficiencies, hypoproliferative disorders, physical trauma, lesions, and wounds; for example, to promote wound healing, or to promote regeneration in degenerated, lesioned or injured tissues, etc.

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5.7 DOSAGE REGIMENS AND FORMULATION

Covalent or noncovalent complexes of $\alpha 2M$ polypeptides and antigenic molecules of the invention may be formulated into pharmaceutical preparations for administration to
35 mammals for treatment or prevention of infectious diseases or cancer at therapeutically effective doses for immunotherapy.

Selection of the preferred effective dose will be determined by a skilled artisan based upon considering several factors which will be known to one of ordinary skill in the art. Such factors include the particular form of $\alpha 2M$, and its pharmacokinetic parameters such as bioavailability, metabolism, half-life, *etc.*, which will have been established during the usual development procedures typically employed in obtaining regulatory approval for a pharmaceutical compound. Further factors in considering the dose include the condition or disease to be treated or the benefit to be achieved in a normal individual, the body mass of the patient, the route of administration, whether administration is acute or chronic, concomitant medications, and other factors well known to affect the efficacy of administered pharmaceutical agents. Thus the precise dosage should be decided according to the judgment of the practitioner and each patient's circumstances, *e.g.*, depending upon the condition and the immune status of the individual patient, according to standard clinical techniques.

Covalent or noncovalent complexes of $\alpha 2M$ polypeptides and antigenic molecules of the invention may be formulated into pharmaceutical preparations for administration to mammals for treatment or prevention of infectious diseases or cancer. Drug solubility and the site of absorption are factors which should be considered when choosing the route of administration of a therapeutic agent.

$\alpha 2M$ polypeptide-antigenic molecule complexes of the invention may optionally be administered with one or more adjuvants in order to enhance the immunological response. For example, depending on the host species, adjuvants which may be used include, but are not limited to: mineral salts or mineral gels such as aluminum hydroxide, aluminum phosphate, and calcium phosphate; surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, and dinitrophenol; immunostimulatory molecules, such as cytokines, saponins, muramyl dipeptides and tripeptide derivatives, CpG dinucleotides, CpG oligonucleotides, monophosphoryl Lipid A, and polyphosphazenes; particulate and microparticulate adjuvant, such as emulsions, liposomes, virosomes, cochleates; or an immune stimulating complex mucosal adjuvants, Freund's (complete and incomplete, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.).

$\alpha 2M$ polypeptide-antigenic molecule complexes of the invention may be administered using any desired route of administration, including but not limited to, *e.g.*, subcutaneously, intravenously or intramuscularly, although intradermally or mucosally is preferred. Advantages of intradermal or mucosal administration include use of lower doses and rapid absorption, respectively. Mucosal routes of administration include, but are not limited to, oral, rectal and nasal administration. Preparations for mucosal administrations are

suitable in various formulations as described below. The route of administration can be varied during a course of treatment.

The doses recited above are preferably given once weekly for a period of about 4-6 weeks, and the mode or site of administration is preferably varied with each administration. In a preferred example, subcutaneous administrations are given, with each site of administration varied sequentially. Thus, by way of example and not limitation, the first injection may be given subcutaneously on the left arm, the second on the right arm, the third on the left belly, the fourth on the right belly, the fifth on the left thigh, the sixth on the right thigh, etc. The same site may be repeated after a gap of one or more injections. Also, split injections may be given. Thus, for example, half the dose may be given in one site and the other half on an other site on the same day.

Alternatively, the mode of administration is sequentially varied, *e.g.*, weekly injections are given in sequence subcutaneously, intramuscularly, intravenously or intraperitoneally.

After 4-6 weeks, further injections are preferably given at two-week intervals over a period of time of one month. Later injections may be given monthly. The pace of later injections may be modified, depending upon the patient's clinical progress and responsiveness to the immunotherapy.

Compositions comprising covalent or noncovalent complexes formulated in a compatible pharmaceutical carrier may be prepared, packaged, and labeled for treatment of the indicated infectious disease or tumor. In preferred aspects, an amount of $\alpha 2M$ polypeptide – antigenic molecule complex is administered to a human that is in the range of about 2 to 150 μg , preferably 2 to 50 μg , most preferably about 25 μg , given once weekly for about 4-6 weeks, intradermally with the site of administration varied sequentially.

If the complex is water-soluble, then it may be formulated in an appropriate buffer, for example, phosphate buffered saline or other physiologically compatible solutions. Alternatively, if the resulting complex has poor solubility in aqueous solvents, then it may be formulated with a non-ionic surfactant such as Tween, or polyethylene glycol. Thus, the covalent or noncovalent complexes and their physiologically acceptable solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, rectal administration or, in the case of tumors, directly injected into a solid tumor.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as

suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

Preparations for oral administration may be suitably formulated to give controlled release of the complexes. Such compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the complexes may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the complexes and a suitable powder base such as lactose or starch.

The complexes may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The complexes may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the complexes may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the complexes may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as

sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophilic drugs.

The complexes may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the covalent or noncovalent complexes. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The invention also provides kits for carrying out the therapeutic regimens of the invention. Such kits comprise in one or more containers therapeutically or prophylactically effective amounts of the covalent or noncovalent $\alpha 2M$ polypeptide – antigenic molecule complexes in pharmaceutically acceptable form. The $\alpha 2M$ polypeptide – antigenic molecule complexes in a vial of a kit of the invention may be in the form of a pharmaceutically acceptable solution, e.g., in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the complex may be lyophilized or desiccated; in this instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (e.g., saline, dextrose solution, etc.), preferably sterile, to reconstitute the complex to form a solution for injection purposes.

In another embodiment, a kit of the invention further comprises a needle or syringe, preferably packaged in sterile form, for injecting the complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of $\alpha 2M$ polypeptide – antigenic molecule complexes by a clinician or by the patient.

6. EXAMPLE: $\alpha 2M$ ANTAGONIZES HSP-MEDIATED ANTIGEN PRESENTATION VIA THE $\alpha 2M$ RECEPTOR

6.1 INTRODUCTION

The Example presented herein describes the successful identification of an interaction between gp96 and the $\alpha 2M$ receptor present in macrophages and dendritic cells *in vivo*, and the blocking of this interaction by $\alpha 2M$. The experiments presented herein form the basis for the compositions and therapeutic methods of the present invention which relate to the use of $\alpha 2M$ polypeptide-antigenic molecule complexes as immunotherapeutic agents for treatment of immune disorders, proliferative disorders, and infectious diseases.

The Applicant of the present invention noted that certain observations were inconsistent with a "direct transfer" model of HSP-chaperoned peptide antigen presentation. First, the immunogenicity of HSP preparations is dependent on the presence of functional phagocytic cells but not B cells or other nonprofessional antigen-presenting cells (Udono and Srivastava, 1993, *supra*; Suto and Srivastava, 1995, *supra*), whereas free peptides can

sensitize all cell types. Second, extremely small quantities of HSP-peptide complexes were effective in eliciting specific immunity, *i.e.*, gp96-chaperoned peptides are several hundred times as effective as free peptides in sensitizing macrophages for CTL recognition, suggesting the possibility of a specific uptake mechanism. Third, gp96-chaperoned peptides elicited an MHC I response that was not limited by the size of peptide. Finally, the processing of gp96-peptide complexes in macrophage was found to be sensitive to Brefeldin A (BFA), which blocks transport through the Golgi apparatus, suggesting that processing occurred through an intercellular mechanism. These observations led to the hypothesis that HSP-chaperoned peptides may be processed internally and re-presented by MHC class I molecules on the cell surfaces of macrophages (Suto and Srivastava, 1995, *supra*). There is also the hypothesis that the mannose receptor is used in the uptake of gp96 but no mechanism has been proposed for the non-glycosylated HSPs, such as HSP70 (Ciupitu *et al.*, 1998, J. Exp. Med., 187: 685-691). Others suggested that a novel intracellular trafficking pathway may be involved for the transport of peptides from the extracellular medium into the lumen of ER) Day *et al.*, 1997, Proc. Natl. Acad. Sci. 94:8065-8069; Nicchitta, 1998, Curr. Opin. in Immunol. 10:103-109). Further suggestions include the involvement of phagocytes which (a) possess an ill-defined pathway to shunt protein from the phagosome into the cytosol where it would enter the normal class I pathway; (b) digest ingested material in lysosomes and regurgitate peptides for loading on the surface to class I molecules (Bevan, 1995, J. Exp. Med. 192:639-41). The discovery of a receptor for heat shock protein as disclosed herein helps to resolve the paradox of how extracellular antigenic peptides complexed to HSPs can be presented by MHC class I molecules on antigen presenting cells.

6.2 MATERIALS AND METHODS

Affinity chromatography. Proteins (1mg) in 2ml volume were incubated with 2ml of equilibrated AminoLink beads in PBS with a reductant (NaCNBH_3) for 1 hour. Uncoupled protein was removed by extensive washing of the column and unreactive groups quenched. Immobilization yields were typically >92% of the starting amount of protein. Columns were stored at 4°C until used. Such columns were made with gp96 (purified as described in Srivastava *et al.*, 1986, Proc. Natl. Acad. Sci., U.S.A. 83:3407-3411) and albumin. For membrane purification, cells were lysed by dounce homogenization in hypotonic buffer containing PMSF. Unlyzed cells and nuclei were removed by centrifugation at 1000g for 5 mm. The postnuclear supernatant was centrifuged at 100,000g for 90 mins. The pellet contains total membranes and was fractionated by aqueous two-phase partition with a dextran/polyethylene glycol biphasic. Briefly membranes were resuspended in PEG (33% wt/wt in 0.22 M sodium phosphate buffer, pH 6.5) and underlaid gently with dextran

(20%wt/wt in 0.22M sodium phosphate buffer, pH 6.5). The two phases were mixed gently and centrifuged at 2000 g for 15 mins. The white material at the interphase was enriched for plasma membranes, whose proteins were extracted by 2 hr incubation in 20mM Tris buffer (pH8, containing 0.08% octylglucoside) at 4°C.

Photo cross-linking of gp96 to putative receptor. The cross-linker (SASD, (Pierce) was labeled with I¹²⁵ using iodobeads (Pierce). Radiolabeled SASD was covalently attached to gp96 by incubation at room temperature for 1 hr. Free SASD and I¹²⁵ were removed by size exclusion column (KwikSep columns, Pierce). For cross-linking studies, I¹²⁵-SASD-gp96 (50 µg gp96) was incubated with purified CD11b⁺ cells. Unbound protein was removed by washing. All procedures to this point were carried out in very dim light. Proteins were cross-linked with UV light. Cells were lysed with lysis buffer (0.5%NP40, 10mM Tris, 1mMEDTA, 150mM NaCl) and treated with 100 mM 2-mercaptoethanol to cleave the cross-linker. *Cell lysates were analyzed by SDS-PAGE and autoradiography.*

Re-presentation assays. Re-presentation assays were carried out as described (Suto and Srivastava, 1995, Science 269:1585-1588). Antigen presenting cells (RAW264.7 macrophage cell line) were plated at a 1:1 ratio with AH I -specific T cells in complete RPMI. Approximately 10,000 cells of each type were used. Gp96 (10 µg/ml) chaperoning the AH1-20 mer peptide (RVTYHSPSYVYHQFERRAK) was added to the cells and the entire culture was incubated for 20 hrs. Stimulation of T cells was measured by quantifying the amount of IFN-γ released into the supernatants by ELISA (Endogen).

Protein Microsequencing. Proteins identified by affinity chromatography were analyzed on SDS-PAGE and stained with coomassie blue or transferred onto PVDF membrane and stained with coomassie blue, all of it under keratin-free conditions. Protein bands were excised with a razor from the gel or membrane. Tryptic peptides from an 80kDa faint coomassie band were extracted by 50% acetonitrile, 5% formic acid, dried, and loaded onto a 75 m 10 cm, reverse-phase C18, microcapillary column (3 µl vol) and tryptic peptides were separated by on-line microcapillary liquid chromatography-tandem mass spectrometry followed by database searching using the SEQUEST program as previously described. (Gatlin *et al.*, 2000, Anal. Chem. 72:757-63; Link *et al.*, 1999, Nat. Biotechnol. 17:676-82). The analysis was carried out in a data-dependent auto-MS/MS fashion using a Finnigan LCQ iontrap Mass Spectrometer.

6.3 RESULTS

Identification of an 80 kDa protein as a potential gp96 receptor. Homogenous preparations of gp96 were coupled to FITC and the gp96-FITC was used to stain RAW264.7 cells, shown to be functionally capable of re-presenting gp96-chaperoned peptides. Gp96-FITC but not control albumin-FITC preparations stained the cell surface of RAW264.7 cells (FIG. 1A). Plasma membrane preparations of cell surface-biotinylated RAW264.7 cells were solubilized in 0.08% octyl-glucoside and the soluble extract was applied to a gp96-Sepharose column. The bound proteins were eluted with 3M sodium chloride. SDS-PAGE analysis of the eluate showed 2 major bands of ~75-80 kDa size (FIG. 1B, top left). Blotting of this gel with avidin-peroxidase showed that both bands were biotinylated, indicating their surface localization (FIG. 1B, bottom left). Affinity purification of membrane extracts of RAW264.7 cells over control serum albumin affinity columns did not result in isolation of any proteins, nor did probing of immunoblots of such gels with avidin peroxidase detect any albumin-binding surface proteins (FIG. 1B, top and bottom center lanes). As an additional control, chromatography of membrane extracts of P815 cells which do not bind gp96-FITC and which do not re-present gp96-chaperoned peptides, on gp96 affinity columns did not result in elution of any gp96-binding proteins (FIG. 1B, top and bottom right lanes).

In parallel experiments, gp96 molecules were coupled to the radio-iodinated linker sulfosuccinimidyl (4-azidosalicylamido) hexanoate (SASD) which contains a photo cross-linkable group. Gp96-SASD- I^{125} was pulsed onto peritoneal macrophages, which have been shown previously to re-present gp96-chaperoned peptides (Suto and Srivastava, 1995, Science 269:1585-1588). Excess gp96-SASD was removed by multiple rounds of washing of the cells and photoactivation was carried out by exposure of cells to UV light for 10 mm. Cell lysates were reduced in order to transfer the I^{125} group to the putative gp96 ligand and were analyzed by SDS- PAGE followed by autoradiography. The gp96 molecule was observed to cross-link to an ~80 kDa band specifically present in re-presentation-competent macrophage but not in the re-presentation-incompetent P815 cells (FIG. 1C). This band appears to correspond in size to the larger of the two bands seen in eluates of gp96 affinity columns (FIG. 1D). No band corresponding to the lower band in that preparation is seen in the photo cross-linked preparation. The observation of a specific binding of gp96 to an 80 kDa protein in two different re-presentation-competent cell types, but not in a re-presentation-incompetent cell line, and by two independent assays supported the candidacy of the 80 kDa molecule for the gp96 receptor.

Antiserum against the 80 kDa protein inhibits re-presentation of a gp96-chaperoned antigenic peptide. The eluates containing the 75-80 kDa proteins were used to immunize a New Zealand white rabbit, and pre-immune and immune sera were used to probe blots of

plasma membrane extracts of the re-presentation-competent RAW264.7 and primary peritoneal macrophages and the re-presentation-incompetent P815 cells. The immune but not the pre-immune serum detected the 80 kDa band (and a faint lower 75 kDa band) in plasma
5 membrane extracts of primary macrophage and the RAW264.7 membranes but not of P815 cells (FIG. 2A). The pre-immune and immune sera were tested in a functional assay for their ability to block re-presentation of gp96-chaperoned peptides. The L^d-restricted epitope AH1 derived from the gp70 antigen of murine colon carcinoma CT26 (Huang *et al.*, 1996, Proc. Natl. Acad. Sci. U.S.A. 93:9730-9735) was used as the model system. Complexes of gp96
10 with an AH1 precursor (used to inhibit direct presentation) were pulsed onto RAW264.7 cells which were used to stimulate a L^d/AH1-specific CD8+ T cell clone. Release of interferon- γ by the clones was measured as a marker of their activation. RAW264.7 cells were able to re-present gp96-chaperoned AH1 precursor effectively in this assay. It was observed that at the highest concentration, the immune sera inhibited re-presentation
15 completely (FIG. 2B). Although the pre-immune serum was ineffective in inhibiting re-presentation as compared to the immune sera, it did inhibit re-presentation significantly at higher concentrations. The significance of this observation became clear later when we determined the identity of the gp96 receptor. Repeated immunizations with the affinity-purified gp96-binding proteins did not result in corresponding increase in antibody titers.

20 *Identification of the 80 kDa protein as an amino terminal fragment of the heavy chain of the α 2M receptor.* The 80 kDa protein eluted from the gp96 affinity column was resolved on SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. The protein band was subjected to in-gel trypsin digestion and mass spectrometry-based protein microsequencing as described in the methods in Section 6.2. Four independent tryptic
25 peptides corresponding to N-terminal region of the α 2-macroglobulin (α 2M) receptor, designated by immunologists as CD91, were identified (FIG. 3C).

α 2M inhibits re-presentation of a gp96-chaperoned antigenic peptide by RAW264.7. α 2M receptor is one of the known natural ligands for the α 2M receptor. Its ability to inhibit re-presentation of gp96-chaperoned antigenic peptide AH1 was tested in the assay described
30 in FIG. 2. α 2M but not control proteins selectin (CD62) or serum albumin was observed to inhibit re-presentation completely and titratably (FIG. 4). This observation was also consistent with the result in FIG. 2 that while the pre-immune serum did not detect an 80 kDa band in plasma membranes of RAW264.7 cells, it did inhibit re-presentation to some degree at high concentrations. Thus, by structural as well as functional criteria, the α 2M
35 receptor was determined to fulfill the criteria essential for a receptor for gp96.

6.4 DISCUSSION

The $\alpha 2M$ receptor, which is also designated CD91, was initially identified as a protein related to the low density lipoprotein (LDL) receptor Related Protein (LRP) (Strickland *et al.*, 1990, J. Biol. Chem. 265:17401-17404; Kristensen *et al.*, 1990, FEBS Lett. 276:151-155). The protein consists of an ~420 kDa α subunit, an 85 kDa β subunit and a 39 kDa tightly associated molecule (RAP). The α and β subunits are encoded by a single transcript of ~15 Kb in size (Van Leuven *et al.*, 1993, Biochim. Biophys. Acta. 1173:71-74). The receptor has been shown to be present in cells of the monocytic lineage and in hepatocytes, fibroblasts and keratinocytes. CD91 has been shown previously to bind the activated form of the plasma glycoprotein $\alpha 2M$, which binds to and inhibits a wide variety of endoproteinases. $\alpha 2M$ receptor also binds to other ligands such as transforming growth factor β (O Connor-McCourt *et al.*, 1987, J. Biol. Chem. 262:14090-14099), platelet-derived growth factor (Huang *et al.*, 1984, Proc. Natl. Acad. Sci. U.S.A. 81:342-346), and fibroblast growth factor (Dennis *et al.*, 1989, J. Biol. Chem. 264:7210-7216). $\alpha 2M$ is thus believed to regulate, and specifically diminish, the activities of its various ligands. Complexed with these various ligands, $\alpha 2M$ binds $\alpha 2M$ receptor on the cell surface and is internalized through receptor-mediated endocytosis. Uptake of $\alpha 2M$ -complexed ligands has been assumed thus far to be the primary function of the $\alpha 2M$ receptor, although a role for it in lipid metabolism is also assumed. $\alpha 2M$ receptor ligands other than $\alpha 2M$, such as tissue-specific plasminogen activator-inhibitor complex (Orth *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:7422-7426) and urokinase-PAI1 complex (Nykjaer *et al.*, 1992, J. Biol. Chem. 267:14543-14546), have been identified. These ligands attest to a role for $\alpha 2M$ receptor in clearing a range of extracellular, plasma products.

The studies reported here show that the heat shock protein gp96 is an additional ligand for the $\alpha 2M$ receptor. The human gp96-coding gene has been mapped previously by us at chromosome 12 (q24.2→q24.3) (Maki *et al.*, 1993, Somatic Cell Mol. Gen. 19:73-81). It is of interest in this regard that the $\alpha 2M$ receptor gene has been mapped to the same chromosome and at a not too distant location (q13→q14) (Hilliker *et al.* Genomics 13:472-474). Gp96 binds $\alpha 2M$ receptor directly and not through other ligands such as $\alpha 2M$. Homogenous preparations of gp96, in solution, or cross-linked to a solid matrix, bind to the $\alpha 2M$ receptor. Indeed, the major ligand for the $\alpha 2M$ receptor, $\alpha 2M$, actually inhibits interaction of gp96 with $\alpha 2M$ receptor, instead of promoting it, providing evidence that gp96 is a direct ligand for the $\alpha 2M$ receptor. The 80 kDa protein shown to bind gp96 is clearly an amino terminal degradation product of the α subunit of the $\alpha 2M$ receptor. Degradation products of the $\alpha 2M$ receptor in this size range have also been observed in previous studies

(Jensen *et al.*, 1989, *Biochem. Arch.* 5:171-176), and may indicate the existence of a discrete ectodomain in the $\alpha 2M$ receptor which may be particularly sensitive to proteolytic cleavage.

As shown here, the gp96- $\alpha 2M$ receptor interaction provides a new type of function for $\alpha 2M$ receptor, a function of a sensor, not only of the extracellular environment with its previously known plasma-based ligands, but also a sensor of the intracellular milieu as well. HSPs such as gp96 are obligate intracellular molecules and are released into the extracellular milieu only under conditions of necrotic (but not apoptotic) cell death. Thus, the $\alpha 2M$ receptor may act as a sensor for necrotic cell death (see FIG. 5), just as the scavenger receptor CD36 and the recently identified phosphatidyl serine-binding protein act as sensors of apoptotic cell death and receptors for apoptotic cells (Savill *et al.*, 1992, *J. Clin. Invest.* 90:1513-1522; Fadok *et al.*, 2000, *Nature* 405:85-90). Interaction of the macrophages with the apoptotic cells leads to a down-regulation of the inflammatory cytokines such as TNF (Fadok *et al.*, 2000, *supra*), while gp96-APC interaction leads to re-presentation of gp96-chaperoned peptides by MHC I molecules of the APC, followed by stimulation of antigen-specific T cells (Suto and Srivastava, 1995, *supra*) and, in addition, secretion of pro-inflammatory cytokines such as TNF, GM-CSF and IL-12. Interestingly, $\alpha 2M$, an independent ligand for the $\alpha 2M$ receptor, inhibits representation of gp96-chaperoned peptides by macrophages. This observation suggests that re-presentation of gp96-chaperoned peptides can not occur physiologically in blood, but only within tissues as a result of localized necrotic cell death. This is consistent with the complete absence of gp96 or other HSPs in blood under all conditions tested. Together, these observations point towards a possible mechanism whereby the release of HSPs in the blood as a result of severe tissue injury and lysis will not lead to a systemic and lethal pro-inflammatory cytokine cascade.

It is possible, therefore, that the $\alpha 2M$ receptor renders it possible for the APCs to sample (i) the extracellular milieu of the blood through $\alpha 2M$ and other plasma ligands and (ii) the intracellular milieu of the tissues through HSPs, particularly of the gp96 family. The former permits APCs to implement their primordial phagocytic function, while the latter allows them to execute its innate and adaptive immunological functions. Viewed in another perspective, recognition of apoptotic cells by APCs through CD36 or phosphatidyl serine, leads to anti-inflammatory signals, while interaction of the APC with necrotic cells through $\alpha 2M$ receptor leads to pro-inflammatory innate and adaptive immune responses (see Srivastava *et al.*, 1998, *Immunity* 8: 657-665).



The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention.

5 Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

10 All references cited herein, including patent applications, patents, and other publications, are incorporated by reference herein in their entireties for all purposes.

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WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an amount of a molecular complex effective for treatment or prevention of an infectious disease or cancer, and a pharmaceutically acceptable carrier, said molecular complex comprising an alpha (2) 5
macroglobulin polypeptide noncovalently associated with an antigenic molecule which displays the antigenicity of an antigen of an infectious agent or an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.
2. The pharmaceutical composition of Claim 1 wherein the antigenic molecule 10
displays the antigenicity of an antigen of an infectious agent.
3. The pharmaceutical composition of Claim 1 wherein the antigenic molecule displays the antigenicity of an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type. 15
4. The pharmaceutical composition of Claim 1 wherein the antigenic molecule is a tumor specific antigen or a tumor-associated antigen.
5. A pharmaceutical composition comprising an amount of a fusion protein 20
effective for treatment or prevention of an infectious disease or cancer, and a pharmaceutically acceptable carrier, said fusion protein comprised of an alpha (2) macroglobulin polypeptide and an antigenic molecule which displays the antigenicity of an antigen of an infectious agent or an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type. 25
6. The pharmaceutical composition of Claim 1 wherein the molecular complex is purified.
7. A purified molecular complex comprising an alpha (2) macroglobulin 30
polypeptide noncovalently associated with an antigenic molecule of an infectious agent or an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.
8. A purified population of molecular complexes in which at least 65% of said 35
complexes comprise an alpha (2) macroglobulin noncovalently associated with an antigenic molecule.

9. A purified population of molecular complexes purified from a recombinant cell in which at least 65% of said complexes comprise an alpha (2) macroglobulin noncovalently associated with an antigenic molecule.

5

10. A recombinant cell infected with a pathogen and transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin polypeptide, which alpha (2) macroglobulin polypeptide associates with an antigenic molecule, when said antigenic molecule is present, to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule.

10

11. A recombinant cancer cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin polypeptide, which alpha (2) macroglobulin polypeptide associates with an antigenic molecule, when said antigen is present, to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule.

15

12. A recombinant cell transformed with (i) a first nucleic acid comprising a first nucleotide sequence that is operably linked to a first promoter and encodes an alpha (2) macroglobulin polypeptide, and (ii) a second nucleic acid comprising a second nucleotide sequence that is operably linked to a second promoter and encodes an antigenic molecule, such that the alpha (2) macroglobulin polypeptide and the antigenic molecule are expressed within the cell and associate with each other to form a complex that in sufficient amount is capable of eliciting an immune response to the antigenic molecule.

25

13. The recombinant cell of Claim 10, 11, or 12 which is a human cell.

14. A pharmaceutical composition comprising the recombinant cell of any one of Claims 10, 11, or 12 and a pharmaceutically acceptable carrier.

30

15. A method for preparing a complex of an alpha (2) macroglobulin polypeptide noncovalently associated with an antigenic molecule, said alpha (2) macroglobulin polypeptide comprising:

35

- (a) culturing a cell transformed with a nucleic acid comprising a nucleotide sequence encoding the alpha (2) macroglobulin polypeptide, under conditions such that said alpha (2) macroglobulin

polypeptide is expressed by the cells and associates with an antigenic molecule of the cell; and

- 5 (b) recovering a population of complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules from the cells.

16. A method for preparing an alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules derived from one or more antigens of an infectious agent, comprising:

- 10 (a) culturing infected cells, transformed with a nucleic acid comprising a nucleotide sequence encoding the alpha (2) macroglobulin polypeptide, or fragment, analog, or variant thereof, and operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the cells and associates with peptides of the cells; and
- 15 (b) recovering from the cells a population of complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with peptides derived from the infectious agent.

20

17. The method of Claim 15 or 16, further comprising purifying the complexes.

18. The method of Claim 15 or 16, further comprising purifying the complexes by affinity chromatography.

25

19. A method of treating or preventing an infectious disease in an individual having an infectious disease comprising administering to the individual an immunogenic amount of purified complexes comprising an alpha (2) macroglobulin polypeptide noncovalently associated with a first antigenic molecule, wherein the first antigenic molecule displays the antigenicity of an antigen of an infectious agent of the infectious disease.

30

20. The method of Claim 19, further comprising, before, concurrently or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second complex of alpha (2) macroglobulin polypeptide noncovalently associated with a second antigenic molecule, said second antigenic molecule displaying the antigenicity of a second antigenic molecule of said infectious agent.

35

21. A method of treating or preventing an infectious disease in a subject having an infectious disease comprising:

- 5 (a) culturing an infected cell transformed with a nucleic acid comprising a nucleotide sequence encoding an alpha (2) macroglobulin polypeptide, said infected cell displaying the antigenicity of an antigen of an infectious agent of the infectious disease, said nucleotide sequence being operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the infected cells and associates with antigenic molecules of the cell;
- 10 (b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with antigenic molecules from the infected cell; and
- 15 (c) administering to the subject an amount of the recovered complexes effective to treat or prevent the infectious disease.

22. The method of Claim 21, further comprising, prior to step (a), the step of obtaining infected cells from the subject and transforming the infected cells with the nucleic acid.

20

23. The method of Claim 21, further comprising, prior to step (a), the step of obtaining the infected cell from one or more individuals and transforming the infected cells with the nucleic acid, said one or more individuals being different from the subject and having the same type of infectious disease as the subject.

25

24. A method of treating or preventing an infectious disease in a subject having an infectious disease comprising:

- 30 (a) culturing a recombinant cell transformed with (i) a first nucleic acid encoding an alpha (2) macroglobulin polypeptide and (ii) a second nucleic acid encoding an antigenic molecule displaying the antigenicity of an antigen of an infectious agent of the infectious disease;
- (b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with the antigen; and
- 35 (c) administering to the subject an amount of the recovered complexes effective to treat or prevent the infectious disease.

25. The method of Claim 19, 21, or 24, in which the infectious disease is caused by an infectious agent selected from the group consisting of a virus, a bacterium, a fungus, and a parasite.

5

26. A method of treating or preventing cancer in an individual having a type of cancer or in whom prevention of a type of cancer is desired comprising administering to the individual an immunogenic amount of purified complexes comprising an alpha (2) macroglobulin polypeptide noncovalently associated with a first antigenic molecule, wherein
10 either (a) the first antigenic molecule displays antigenicity of said type of cancer or a metastasis thereof; or (b) the complex is obtained by recovering complexes from said type of cancer cells or a metastasis thereof that recombinantly express the alpha (2) macroglobulin polypeptide.

15

27. The method of Claim 26, further comprising, before, concurrently or after administration of the immunogenic complex, administering to the individual a composition comprising antigen presenting cells sensitized *in vitro* with a sensitizing amount of a second complex of an alpha (2) macroglobulin noncovalently associated with a second antigenic molecule, said second antigenic molecule displaying the antigenicity of an antigen
20 overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.

28. The method of Claim 26, wherein the first antigenic molecule is an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell
25 type.

29. The pharmaceutical composition of Claim 26, wherein the antigenic molecule is a tumor-specific antigen or a tumor-associated antigen.

30

30. A method of treating or preventing cancer in a subject having a type of cancer or in whom prevention of a type of cancer is desired comprising:

35

- (a) culturing a cancer cell of said type transformed with a nucleic acid comprising a nucleotide sequence encoding an alpha (2) macroglobulin polypeptide said nucleotide sequence being operably linked to a promoter, under conditions such that the alpha (2) macroglobulin polypeptide is expressed by the cancer cell and associates with at least one antigenic molecule of the cell;

- (b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with at least one antigenic molecule from the cancer cell; and
- 5 (c) administering to the subject an amount of the recovered complexes effective to treat or prevent cancer.

31. The method of Claim 30, further comprising, prior to step (a), the step of obtaining cancer cells from the subject and transforming the cancer cells with the nucleic
10 acid.

32. The method of Claim 30, further comprising, prior to step (a), the step of obtaining cancer cells from one or more individuals and transforming the cancer cells with the nucleic acid, said one or more individuals being different from the subject and having the
15 same type of cancer as the subject.

33. A method of treating or preventing cancer in a subject having a type of cancer or in whom prevention of a type of cancer is desired comprising:

- 20 (a) culturing a recombinant cell transformed with (i) a first nucleic acid encoding an alpha (2) macroglobulin polypeptide, and (ii) a second nucleic acid encoding an antigenic molecule displaying the antigenicity of an antigen of a cancer cell;
- (b) recovering complexes of the alpha (2) macroglobulin polypeptide noncovalently associated with the antigen; and
- 25 (c) administering to the subject an amount of the recovered complexes effective to treat or prevent cancer.

34. The method of Claim 26, 30, or 33, in which the type of cancer is selected from the group consisting of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary
35 adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung

carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia,
5 lymphoma, multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

35. A method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment an antibody specific for alpha (2) macroglobulin.

10 36. The method of Claim 35, wherein the antibody is purified.

15

20

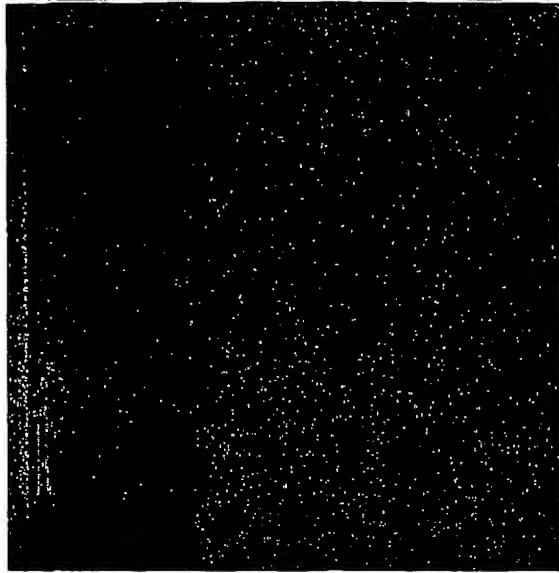
25

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FITC-SA



FITC-gp96

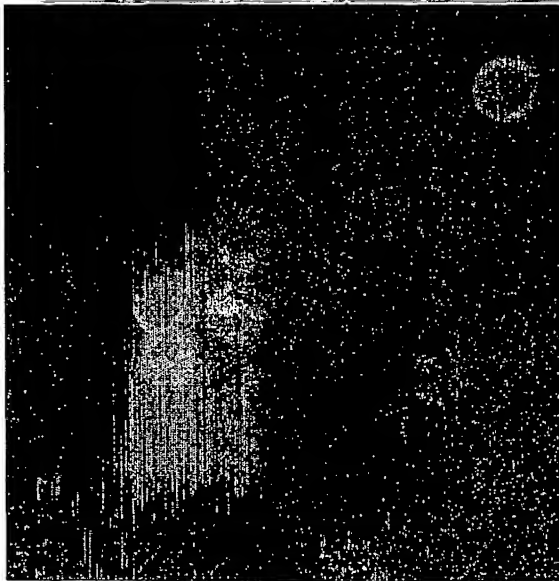


FIG. 1A

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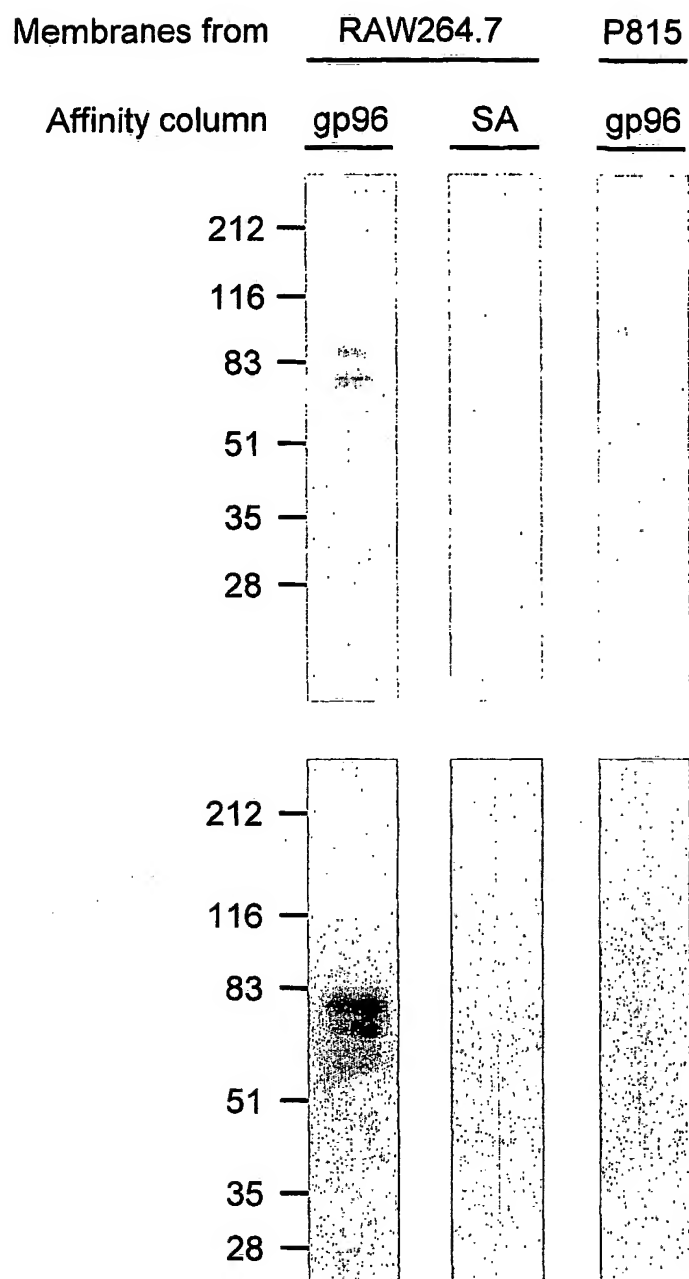


FIG.1B

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CELL	MO	MO	MO	P815
^{125}I -SASD-gp96	+	+	+	+
UV	+	-	+	+
2-ME	+	+	-	+

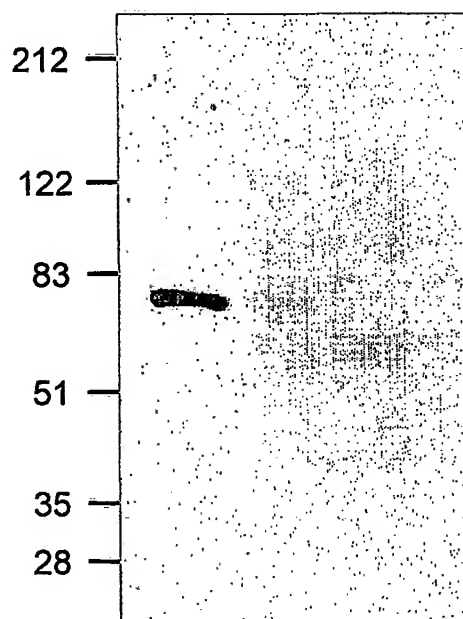


FIG.1C

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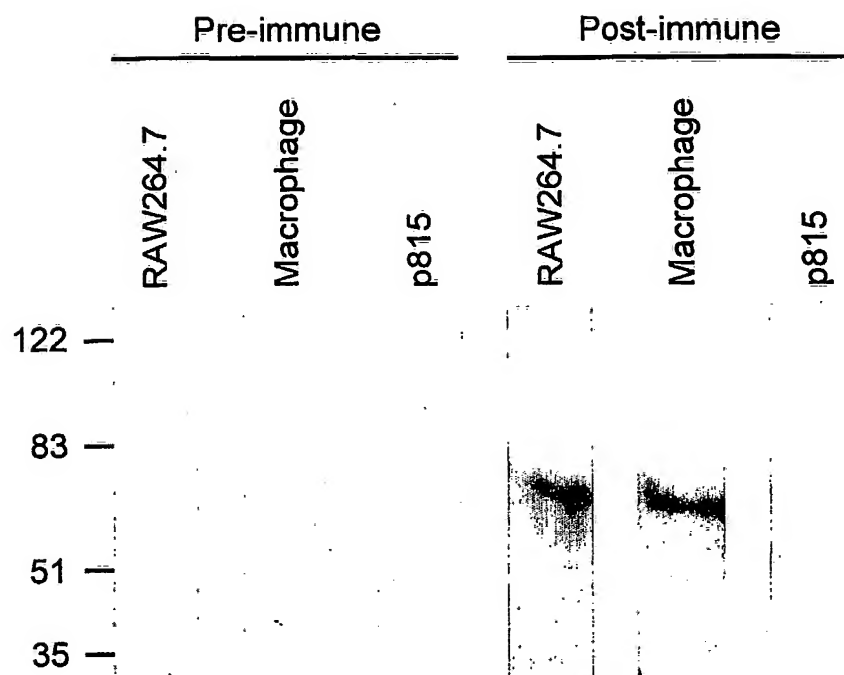


FIG.2A

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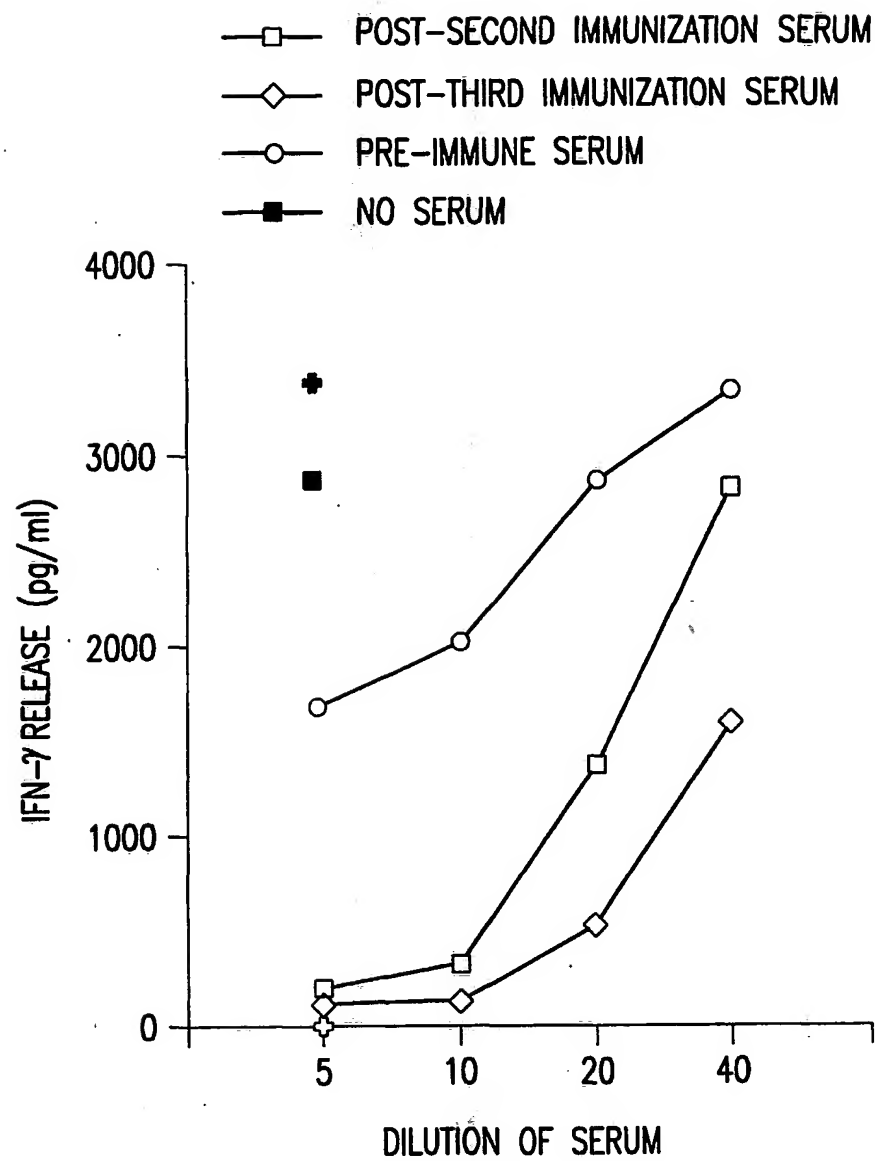


FIG.2B

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<u>Seq</u>	<u>#</u>	<u>b</u>	<u>y</u>	<u>+1</u>
G	1	58.1	-	10
G	2	115.1	1095.2	9
A	3	186.2	1038.2	8
L	4	299.3	967.1	7
H	5	436.5	853.9	6
I	6	549.6	716.8	5
Y	7	712.8	603.6	4
H	8	850.0	440.5	3
Q	9	978.1	303.3	2
R	10	-	175.2	1

FIG.3A

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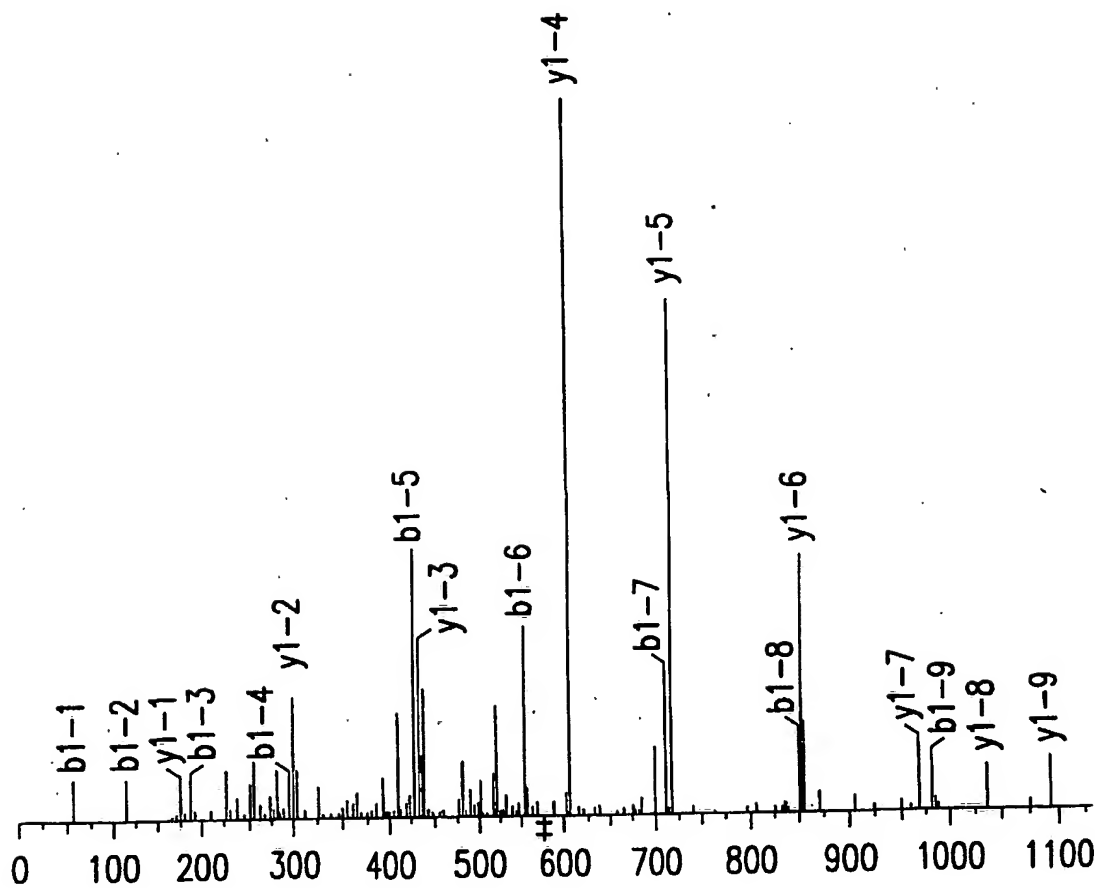


FIG.3B

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POSITION	MH+	SEQUENCE	
509-518	955.0122	SGFSLGSDGK	(SEQ ID NO: 54)
328-337	973.1753	GIALDPAMGK	(SEQ ID NO: 55)
460-469	1152.3010	GGALHIYHQR	(SEQ ID NO: 56)
338-348	1315.5116	VFFTDYGQIPK	(SEQ ID NO: 57)

FIG.3C

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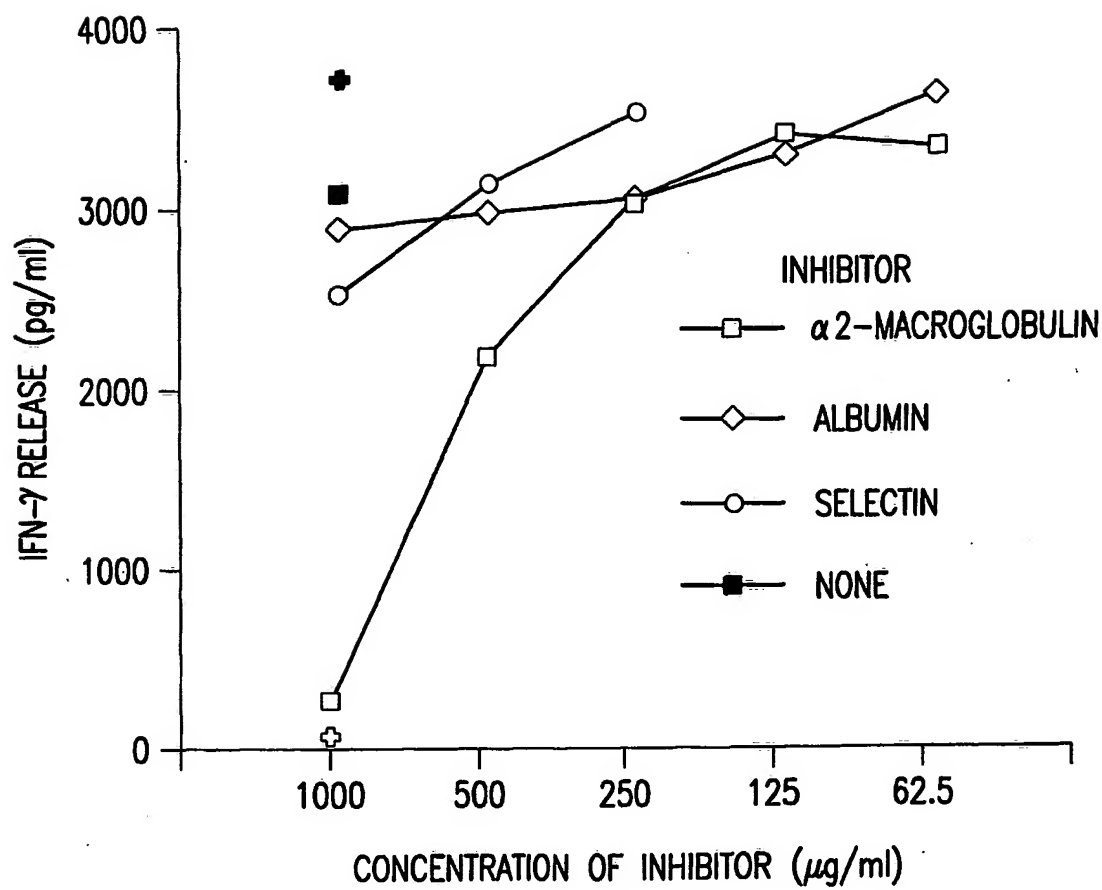


FIG.4

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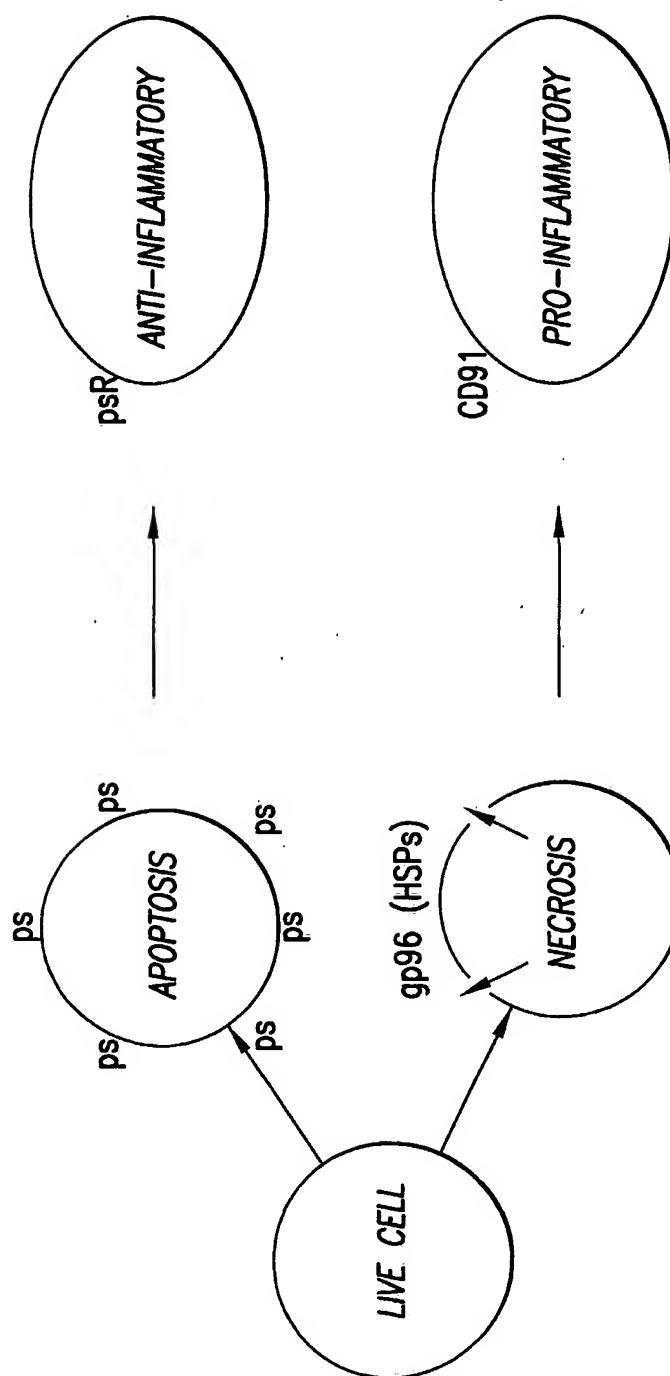


FIG.5

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CGCTGCTCCC	CGCCAGTGCA	CTGAGGAGGC	GGAAACGGGG	GAGCCCCTAG	TGCTCCATCA	60										
GGCCCCTACC	AAGGCACCCC	CATCGGGTCC	ACGCCCCCCA	CCCCCACC	CGCCTCCTCC	120										
CAATTGTGCA	TTTTTGCAGC	CGGAGTCGGC	TCCGAGATGG	GGCTGTGAGC	TTCGCCCTGG	180										
GAGGGGGAGA	GGAGCGAGGA	GTAAAGCAGG	GGTGAAGGGT	TCGAATTTGG	GGGCAGGGGG	240										
CGCACCCGCG	TCAGCAGGCC	CTTCCCAGGG	GGCTCGGAAC	TGTACCATT	CACCTATGCC	300										
CCTGGTTTCG	TTTGCTTAAG	GAAGGATAAG	ATAGAAGAGT	CGGGGAGAGG	AAGATAAAGG	360										
GGGACCCCCC	AATTGGGGGG	GGCGAGGACA	AGAAGTAACA	GGACCAGAGG	GTGGGGGCTG	420										
CTGTTTGCAT	CGGCCACAC	C	ATG	CTG	ACC	CCG	CCG	TTG	CTG	CTG	CTC	GTG	471			
		Met	Leu	Thr	Pro	Pro	Leu	Leu	Leu	Leu	Val					
		1				5						10				
CCG	CTG	CTT	TCA	GCT	CTG	GTC	TCC	GGG	GCC	ACT	ATG	GAT	GCC	CCT	AAA	519
Pro	Leu	Leu	Ser	Ala	Leu	Val	Ser	Gly	Ala	Thr	Met	Asp	Ala	Pro	Lys	
			15						20					25		
ACT	TGC	AGC	CCT	AAG	CAG	TTT	GCC	TGC	AGA	GAC	CAA	ATC	ACC	TGT	ATC	567
Thr	Cys	Ser	Pro	Lys	Gln	Phe	Ala	Cys	Arg	Asp	Gln	Ile	Thr	Cys	Ile	
			30					35					40			
TCA	AAG	GGC	TGG	CGG	TGT	GAC	GGT	GAA	AGA	GAT	TGC	CCC	GAC	GGC	TCT	615
Ser	Lys	Gly	Trp	Arg	Cys	Asp	Gly	Glu	Arg	Asp	Cys	Pro	Asp	Gly	Ser	
		45					50					55				
GAT	GAA	GCC	CCT	GAG	ATC	TGT	CCA	CAG	AGT	AAA	GCC	CAG	AGA	TGC	CCG	663
Asp	Glu	Ala	Pro	Glu	Ile	Cys	Pro	Gln	Ser	Lys	Ala	Gln	Arg	Cys	Pro	
	60					65					70					
CCA	AAT	GAG	CAC	AGT	TGT	CTG	GGG	ACT	GAG	CTA	TGT	GTC	CCC	ATG	TCT	711
Pro	Asn	Glu	His	Ser	Cys	Leu	Gly	Thr	Glu	Leu	Cys	Val	Pro	Met	Ser	
	75				80					85				90		
CGT	CTC	TGC	AAC	GGG	ATC	CAG	GAC	TGC	ATG	GAT	GGC	TCA	GAC	GAG	GGT	759
Arg	Leu	Cys	Asn	Gly	Ile	Gln	Asp	Cys	Met	Asp	Gly	Ser	Asp	Glu	Gly	
			95					100						105		
GCT	CAC	TGC	CGA	GAG	CTC	CGA	GCC	AAC	TGT	TCT	CGA	ATG	GGT	TGT	CAA	807
Ala	His	Cys	Arg	Glu	Leu	Arg	Ala	Asn	Cys	Ser	Arg	Met	Gly	Cys	Gln	
			110					115					120			
CAC	CAT	TGT	GTA	CCT	ACA	CCC	AGT	GGG	CCC	ACG	TGC	TAC	TGT	AAC	AGC	855
His	His	Cys	Val	Pro	Thr	Pro	Ser	Gly	Pro	Thr	Cys	Tyr	Cys	Asn	Ser	
		125					130					135				

FIG.6A-1

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AGC TTC CAG CTC GAG GCA GAT GGC AAG ACG TGC AAA GAT TTT GAC GAG	903
Ser Phe Gln Leu Glu Ala Asp Gly Lys Thr Cys Lys Asp Phe Asp Glu	
140 145 150	
TGT TCC GTG TAT GGC ACC TGC AGC CAG CTT TGC ACC AAC ACA GAT GGC	951
Cys Ser Val Tyr Gly Thr Cys Ser Gln Leu Cys Thr Asn Thr Asp Gly	
155 160 165 170	
TCC TTC ACA TGT GGC TGT GTT GAA GGC TAC CTG CTG CAA CCG GAC AAC	999
Ser Phe Thr Cys Gly Cys Val Glu Gly Tyr Leu Leu Gln Pro Asp Asn	
175 180 185	
CGC TCC TGC AAG GCC AAG AAT GAG CCA GTA GAT CGG CCG CCA GTG CTA	1047
Arg Ser Cys Lys Ala Lys Asn Glu Pro Val Asp Arg Pro Pro Val Leu	
190 195 200	
CTG ATT GCC AAC TCT CAG AAC ATC CTA GCT ACG TAC CTG AGT GGG GCC	1095
Leu Ile Ala Asn Ser Gln Asn Ile Leu Ala Thr Tyr Leu Ser Gly Ala	
205 210 215	
CAA GTG TCT ACC ATC ACA CCC ACC AGC ACC CGA CAA ACC ACG GCC ATG	1143
Gln Val Ser Thr Ile Thr Pro Thr Ser Thr Arg Gln Thr Thr Ala Met	
220 225 230	
GAC TTC AGT TAT GCC AAT GAG ACC GTA TGC TGG GTG CAC GTT GGG GAC	1191
Asp Phe Ser Tyr Ala Asn Glu Thr Val Cys Trp Val His Val Gly Asp	
235 240 245 250	
AGT GCT GCC CAG ACA CAG CTC AAG TGT GCC CGG ATG CCT GGC CTG AAG	1239
Ser Ala Ala Gln Thr Gln Leu Lys Cys Ala Arg Met Pro Gly Leu Lys	
255 260 265	
GGC TTT GTG GAT GAG CAT ACC ATC AAC ATC TCC CTC AGC CTG CAC CAC	1287
Gly Phe Val Asp Glu His Thr Ile Asn Ile Ser Leu Ser Leu His His	
270 275 280	
GTG GAG CAG ATG GCA ATC GAC TGG CTG ACG GGA AAC TTC TAC TTT GTC	1335
Val Glu Gln Met Ala Ile Asp Trp Leu Thr Gly Asn Phe Tyr Phe Val	
285 290 295	
GAC GAC ATT GAC GAC AGG ATC TTT GTC TGT AAC CGA AAC GGG GAC ACC	1383
Asp Asp Ile Asp Asp Arg Ile Phe Val Cys Asn Arg Asn Gly Asp Thr	
300 305 310	

FIG.6A-2

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TGT GTC ACT CTG CTG GAC CTG GAA CTC TAC AAC CCC AAA GGC ATC GCC Cys Val Thr Leu Leu Asp Leu Glu Leu Tyr Asn Pro Lys Gly Ile Ala 315 320 325 330	1431
TTG GAC CCC GCC ATG GGG AAG GTG TTC TTC ACT GAC TAC GGG CAG ATC Leu Asp Pro Ala Met Gly Lys Val Phe Phe Thr Asp Tyr Gly Gln Ile 335 340 345	1479
CCA AAG GTG GAG CGC TGT GAC ATG GAT GGA CAG AAC CGC ACC AAG CTG Pro Lys Val Glu Arg Cys Asp Met Asp Gly Gln Asn Arg Thr Lys Leu 350 355 360	1527
GTG GAT AGC AAG ATC GTG TTT CCA CAC GGC ATC ACC CTG GAC CTG GTC Val Asp Ser Lys Ile Val Phe Pro His Gly Ile Thr Leu Asp Leu Val 365 370 375	1575
AGC CGC CTC GTC TAC TGG GCG GAC GCC TAC CTA GAC TAC ATC GAG GTG Ser Arg Leu Val Tyr Trp Ala Asp Ala Tyr Leu Asp Tyr Ile Glu Val 380 385 390	1623
GTA GAC TAC GAA GGG AAG GGT CGG CAG ACC ATC ATC CAA GGC ATC CTG Val Asp Tyr Glu Gly Lys Gly Arg Gln Thr Ile Ile Gln Gly Ile Leu 395 400 405 410	1671
ATC GAG CAC CTG TAC GGC CTG ACC GTG TTT GAG AAC TAT CTC TAC GCC Ile Glu His Leu Tyr Gly Leu Thr Val Phe Glu Asn Tyr Leu Tyr Ala 415 420 425	1719
ACC AAC TCG GAC AAT GCC AAC ACG CAG CAG AAG ACG AGC GTG ATC CGA Thr Asn Ser Asp Asn Ala Asn Thr Gln Gln Lys Thr Ser Val Ile Arg 430 435 440	1767
GTG AAC CGG TTC AAC AGT ACT GAG TAC CAG GTC GTC ACC CGT GTG GAC Val Asn Arg Phe Asn Ser Thr Glu Tyr Gln Val Val Thr Arg Val Asp 445 450 455	1815
AAG GGT GGT GCC CTG CAT ATC TAC CAC CAG CGA CGC CAG CCC CGA GTG Lys Gly Gly Ala Leu His Ile Tyr His Gln Arg Arg Gln Pro Arg Val 460 465 470	1863
CGG AGT CAC GCC TGT GAG AAT GAC CAG TAC GGG AAG CCA GGT GGC TGC Arg Ser His Ala Cys Glu Asn Asp Gln Tyr Gly Lys Pro Gly Gly Cys 475 480 485 490	1911

FIG.6A-3

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TCC GAC ATC TGC CTC CTG GCC AAC AGT CAC AAG GCA AGG ACC TGC AGG	1959
Ser Asp Ile Cys Leu Leu Ala Asn Ser His Lys Ala Arg Thr Cys Arg	
495 500 505	
TGC AGG TCT GGC TTC AGC CTG GGA AGT GAT GGG AAG TCT TGT AAG AAA	2007
Cys Arg Ser Gly Phe Ser Leu Gly Ser Asp Gly Lys Ser Cys Lys Lys	
510 515 520	
CCT GAA CAT GAG CTG TTC CTC GTG TAT GGC AAG GGC CGA CCA GGC ATC	2055
Pro Glu His Glu Leu Phe Leu Val Tyr Gly Lys Gly Arg Pro Gly Ile	
525 530 535	
ATT AGA GGC ATG GAC ATG GGG GCC AAG GTC CCA GAT GAG CAC ATG ATC	2103
Ile Arg Gly Met Asp Met Gly Ala Lys Val Pro Asp Glu His Met Ile	
540 545 550	
CCC ATC GAG AAC CTT ATG AAT CCA CGC GCT CTG GAC TTC CAC GCC GAG	2151
Pro Ile Glu Asn Leu Met Asn Pro Arg Ala Leu Asp Phe His Ala Glu	
555 560 565 570	
ACC GGC TTC ATC TAC TTT GCT GAC ACC ACC AGC TAC CTC ATT GGC CGC	2199
Thr Gly Phe Ile Tyr Phe Ala Asp Thr Thr Ser Tyr Leu Ile Gly Arg	
575 580 585	
CAG AAA ATT GAT GGC ACG GAG AGA GAG ACT ATC CTG AAG GAT GGC ATC	2247
Gln Lys Ile Asp Gly Thr Glu Arg Glu Thr Ile Leu Lys Asp Gly Ile	
590 595 600	
CAC AAT GTG GAG GGC GTA GCC GTG GAC TGG ATG GGA GAC AAT CTT TAC	2295
His Asn Val Glu Gly Val Ala Val Asp Trp Met Gly Asp Asn Leu Tyr	
605 610 615	
TGG ACT GAT GAT GGC CCC AAG AAG ACC ATT AGT GTG GCC AGG CTG GAG	2343
Trp Thr Asp Asp Gly Pro Lys Lys Thr Ile Ser Val Ala Arg Leu Glu	
620 625 630	
AAA GCC GCT CAG ACC CGG AAG ACT CTA ATT GAG GGC AAG ATG ACA CAC	2391
Lys Ala Ala Gln Thr Arg Lys Thr Leu Ile Glu Gly Lys Met Thr His	
635 640 645 650	
CCC AGG GCC ATT GTA GTG GAT CCA CTC AAT GGG TGG ATG TAC TGG ACA	2439
Pro Arg Ala Ile Val Val Asp Pro Leu Asn Gly Trp Met Tyr Trp Thr	
655 660 665	

FIG.6A-4

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GAC TGG GAG GAG GAC CCC AAG GAC AGT CGG CGA GGG CGG CTC GAG AGG	2487
Asp Trp Glu Glu Asp Pro Lys Asp Ser Arg Arg Gly Arg Leu Glu Arg	
670 675 680	
GCT TGG ATG GAC GGC TCA CAC CGA GAT ATC TTT GTC ACC TCC AAG ACA	2535
Ala Trp Met Asp Gly Ser His Arg Asp Ile Phe Val Thr Ser Lys Thr	
685 690 695	
GTG CTT TGG CCC AAT GGG CTA AGC CTG GAT ATC CCA GCC GGA CGC CTC	2583
Val Leu Trp Pro Asn Gly Leu Ser Leu Asp Ile Pro Ala Gly Arg Leu	
700 705 710	
TAC TGG GTG GAT GCC TTC TAT GAC CGA ATT GAG ACC ATA CTG CTC AAT	2631
Tyr Trp Val Asp Ala Phe Tyr Asp Arg Ile Glu Thr Ile Leu Leu Asn	
715 720 725 730	
GGC ACA GAC CGG AAG ATT GTA TAT GAG GGT CCT GAA CTG AAT CAT GCC	2679
Gly Thr Asp Arg Lys Ile Val Tyr Glu Gly Pro Glu Leu Asn His Ala	
735 740 745	
TTC GGC CTG TGT CAC CAT GGC AAC TAC CTC TTT TGG ACC GAG TAC CGG	2727
Phe Gly Leu Cys His His Gly Asn Tyr Leu Phe Trp Thr Glu Tyr Arg	
750 755 760	
AGC GGC AGC GTC TAC CGC TTG GAA CGG GGC GTG GCA GGC GCA CCG CCC	2775
Ser Gly Ser Val Tyr Arg Leu Glu Arg Gly Val Ala Gly Ala Pro Pro	
765 770 775	
ACT GTG ACC CTT CTG CGC AGC GAG AGA CCG CCT ATC TTT GAG ATC CGA	2823
Thr Val Thr Leu Leu Arg Ser Glu Arg Pro Pro Ile Phe Glu Ile Arg	
780 785 790	
ATG TAC GAC GCG CAC GAG CAG CAA GTG GGT ACC AAC AAA TGC CGG GTA	2871
Met Tyr Asp Ala His Glu Gln Gln Val Gly Thr Asn Lys Cys Arg Val	
795 800 805 810	
AAT AAC GGA GGC TGC AGC AGC CTG TGC CTC GCC ACC CCC GGG AGC CGC	2919
Asn Asn Gly Gly Cys Ser Ser Leu Cys Leu Ala Thr Pro Gly Ser Arg	
815 820 825	
CAG TGT GCC TGT GCC GAG GAC CAG GTG TTG GAC ACA GAT GGT GTC ACC	2967
Gln Cys Ala Cys Ala Glu Asp Gln Val Leu Asp Thr Asp Gly Val Thr	
830 835 840	

FIG.6A-5

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TGC TTG GCG AAC CCA TCC TAC GTG CCC CCA CCC CAG TGC CAG CCG GGC	3015
Cys Leu Ala Asn Pro Ser Tyr Val Pro Pro Pro Gln Cys Gln Pro Gly	
845 850 855	
CAG TTT GCC TGT GCC AAC AAC CGC TGC ATC CAG GAG CGC TGG AAG TGT	3063
Gln Phe Ala Cys Ala Asn Asn Arg Cys Ile Gln Glu Arg Trp Lys Cys	
860 865 870	
GAC GGA GAC AAC GAC TGT CTG GAC AAC AGC GAT GAG GCC CCA GCA CTG	3111
Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp Glu Ala Pro Ala Leu	
875 880 885 890	
TGC CAT CAA CAC ACC TGT CCC TCG GAC CGA TTC AAG TGT GAG AAC AAC	3159
Cys His Gln His Thr Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn	
895 900 905	
CGG TGT ATC CCC AAC CGC TGG CTC TGT GAT GGG GAT AAT GAT TGT GGC	3207
Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly	
910 915 920	
AAC AGC GAG GAC GAA TCC AAT GCC ACG TGC TCA GCC CGC ACC TGT CCA	3255
Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro	
925 930 935	
CCC AAC CAG TTC TCC TGT GCC AGT GGC CGA TGC ATT CCT ATC TCA TGG	3303
Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp	
940 945 950	
ACC TGT GAT CTG GAT GAT GAC TGT GGG GAC CGG TCC GAT GAG TCA GCC	3351
Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala	
955 960 965 970	
TCA TGC GCC TAC CCC ACC TGC TTC CCC CTG ACT CAA TTT ACC TGC AAC	3399
Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn	
975 980 985	
AAT GGC AGA TGT ATT AAC ATC AAC TGG CGG TGT GAC AAC GAC AAT GAC	3447
Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp	
990 995 1000	
TGT GGG GAC AAC AGC GAC GAA GCC GGC TGC AGT CAC TCC TGC TCC AGT	3495
Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser	
1005 1010 1015	

FIG.6A-6

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ACC CAG TTC AAG TGC AAC AGT GGC AGA TGC ATC CCC GAG CAC TGG ACG 3543
 Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile Pro Glu His Trp Thr
 1020 1025 1030

TGT GAT GGG GAC AAT GAT TGT GGG GAC TAC AGC GAC GAG ACA CAC GCC 3591
 Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala
 1035 1040 1045 1050

AAC TGT ACC AAC CAG GCT ACA AGA CCT CCT GGT GGC TGC CAC TCG GAT 3639
 Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly Gly Cys His Ser Asp
 1055 1060 1065

GAG TTC CAG TGC CCG CTA GAT GGC CTG TGC ATC CCC CTG AGG TGG CGC 3687
 Glu Phe Gln Cys Pro Leu Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg
 1070 1075 1080

TGC GAC GGG GAC ACC GAC TGC ATG GAT TCC AGC GAT GAG AAG AGC TGT 3735
 Cys Asp Gly Asp Thr Asp Cys Met Asp Ser Ser Asp Glu Lys Ser Cys
 1085 1090 1095

GAG GGC GTG ACC CAT GTT TGT GAC CCG AAT GTC AAG TTT GGC TGC AAG 3783
 Glu Gly Val Thr His Val Cys Asp Pro Asn Val Lys Phe Gly Cys Lys
 1100 1105 1110

GAC TCC GCC CGG TGC ATC AGC AAG GCG TGG GTG TGT GAT GGC GAC AGC 3831
 Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val Cys Asp Gly Asp Ser
 1115 1120 1125 1130

GAC TGT GAA GAT AAC TCC GAC GAG GAG AAC TGT GAG GCC CTG GCC TGC 3879
 Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys Glu Ala Leu Ala Cys
 1135 1140 1145

AGG CCA CCC TCC CAT CCC TGC GCC AAC AAC ACC TCT GTC TGC CTG CCT 3927
 Arg Pro Pro Ser His Pro Cys Ala Asn Asn Thr Ser Val Cys Leu Pro
 1150 1155 1160

CCT GAC AAG CTG TGC GAC GGC AAG GAT GAC TGT GGA GAC GGC TCG GAT 3975
 Pro Asp Lys Leu Cys Asp Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp
 1165 1170 1175

GAG GGC GAG CTC TGT GAC CAG TGT TCT CTG AAT AAT GGT GGC TGT AGT 4023
 Glu Gly Glu Leu Cys Asp Gln Cys Ser Leu Asn Asn Gly Gly Cys Ser
 1180 1185 1190

FIG.6A-7

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CAC AAC TGC TCA GTG GCC CCT GGT GAA GGC ATC GTG TGC TCT TGC CCT His Asn Cys Ser Val Ala Pro Gly Glu Gly Ile Val Cys Ser Cys Pro 1195 1200 1205 1210	4071
CTG GGC ATG GAG CTG GGC TCT GAC AAC CAC ACC TGC CAG ATC CAG AGC Leu Gly Met Glu Leu Gly Ser Asp Asn His Thr Cys Gln Ile Gln Ser 1215 1220 1225	4119
TAC TGT GCC AAG CAC CTC AAA TGC AGC CAG AAG TGT GAC CAG AAC AAG Tyr Cys Ala Lys His Leu Lys Cys Ser Gln Lys Cys Asp Gln Asn Lys 1230 1235 1240	4167
TTC AGT GTG AAG TGC TCC TGC TAC GAG GGC TGG GTC TTG GAG CCT GAC Phe Ser Val Lys Cys Ser Cys Tyr Glu Gly Trp Val Leu Glu Pro Asp 1245 1250 1255	4215
GGG GAA ACG TGC CGC AGT CTG GAT CCC TTC AAA CTG TTC ATC ATC TTC Gly Glu Thr Cys Arg Ser Leu Asp Pro Phe Lys Leu Phe Ile Ile Phe 1260 1265 1270	4263
TCC AAC CGC CAC GAG ATC AGG CGC ATT GAC CTT CAC AAG GGG GAC TAC Ser Asn Arg His Glu Ile Arg Arg Ile Asp Leu His Lys Gly Asp Tyr 1275 1280 1285 1290	4311
AGC GTC CTA GTG CCT GGC CTG CGC AAC ACT ATT GCC CTG GAC TTC CAC Ser Val Leu Val Pro Gly Leu Arg Asn Thr Ile Ala Leu Asp Phe His 1295 1300 1305	4359
CTC AGC CAG AGT GCC CTC TAC TGG ACC GAC GCG GTA GAG GAC AAG ATC Leu Ser Gln Ser Ala Leu Tyr Trp Thr Asp Ala Val Glu Asp Lys Ile 1310 1315 1320	4407
TAC CGT GGG AAA CTC CTG GAC AAC GGA GCC CTG ACC AGC TTT GAG GTG Tyr Arg Gly Lys Leu Leu Asp Asn Gly Ala Leu Thr Ser Phe Glu Val 1325 1330 1335	4455
GTG ATT CAG TAT GGC TTG GCC ACA CCA GAG GGC CTG GCT GTA GAT TGG Val Ile Gln Tyr Gly Leu Ala Thr Pro Glu Gly Leu Ala Val Asp Trp 1340 1345 1350	4503
ATT GCA GGC AAC ATC TAC TGG GTG GAG AGC AAC CTG GAC CAG ATC GAA Ile Ala Gly Asn Ile Tyr Trp Val Glu Ser Asn Leu Asp Gln Ile Glu 1355 1360 1365 1370	4551

FIG.6A-8

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GTG GCC AAG CTG GAC GGA ACC CTC CGA ACC ACT CTG CTG GCG GGT GAC Val Ala Lys Leu Asp Gly Thr Leu Arg Thr Thr Leu Leu Ala Gly Asp 1375 1380 1385	4599
ATT GAG CAC CCG AGG GCC ATC GCT CTG GAC CCT CGG GAT GGG ATT CTG Ile Glu His Pro Arg Ala Ile Ala Leu Asp Pro Arg Asp Gly Ile Leu 1390 1395 1400	4647
TTT TGG ACA GAC TGG GAT GCC AGC CTG CCA CGA ATC GAG GCT GCA TCC Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro Arg Ile Glu Ala Ala Ser 1405 1410 1415	4695
ATG AGT GGA GCT GGC CGC CGA ACC ATC CAC CGG GAG ACA GGC TCT GGG Met Ser Gly Ala Gly Arg Arg Thr Ile His Arg Glu Thr Gly Ser Gly 1420 1425 1430	4743
GGC TGC GCC AAT GGG CTC ACC GTG GAT TAC CTG GAG AAG CGC ATC CTC Gly Cys Ala Asn Gly Leu Thr Val Asp Tyr Leu Glu Lys Arg Ile Leu 1435 1440 1445 1450	4791
TGG ATT GAT GCT AGG TCA GAT GCC ATC TAT TCA GCC CGG TAT GAC GGC Trp Ile Asp Ala Arg Ser Asp Ala Ile Tyr Ser Ala Arg Tyr Asp Gly 1455 1460 1465	4839
TCC GGC CAC ATG GAG GTG CTT CGG GGA CAC GAG TTC CTG TCA CAC CCA Ser Gly His Met Glu Val Leu Arg Gly His Glu Phe Leu Ser His Pro 1470 1475 1480	4887
TTT GCC GTG ACA CTG TAC GGT GGG GAG GTG TAC TGG ACC GAC TGG CGA Phe Ala Val Thr Leu Tyr Gly Gly Glu Val Tyr Trp Thr Asp Trp Arg 1485 1490 1495	4935
ACA AAT ACA CTG GCT AAG GCC AAC AAG TGG ACT GGC CAC AAC GTC ACC Thr Asn Thr Leu Ala Lys Ala Asn Lys Trp Thr Gly His Asn Val Thr 1500 1505 1510	4983
GTG GTA CAG AGG ACC AAC ACC CAG CCC TTC GAC CTG CAG GTG TAT CAC Val Val Gln Arg Thr Asn Thr Gln Pro Phe Asp Leu Gln Val Tyr His 1515 1520 1525 1530	5031
CCT TCC CGG CAG CCC ATG GCT CCA AAC CCA TGT GAG GCC AAT GGC GGC Pro Ser Arg Gln Pro Met Ala Pro Asn Pro Cys Glu Ala Asn Gly Gly 1535 1540 1545	5079

FIG. 6A-9

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CGG GGC CCC TGT TCC CAT CTG TGC CTC ATC AAC TAC AAC CGG ACC GTC Arg Gly Pro Cys Ser His Leu Cys Leu Ile Asn Tyr Asn Arg Thr Val 1550 1555 1560	5127
TCC TGG GCC TGT CCC CAC CTC ATG AAG CTG CAC AAG GAC AAC ACC ACC Ser Trp Ala Cys Pro His Leu Met Lys Leu His Lys Asp Asn Thr Thr 1565 1570 1575	5175
TGC TAT GAG TTT AAG AAG TTC CTG CTG TAC GCA CGT CAG ATG GAG ATC Cys Tyr Glu Phe Lys Lys Phe Leu Leu Tyr Ala Arg Gln Met Glu Ile 1580 1585 1590	5223
CGG GGC GTG GAC CTG GAT GCC CCG TAC TAC AAT TAT ATC ATC TCC TTC Arg Gly Val Asp Leu Asp Ala Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe 1595 1600 1605 1610	5271
ACG GTG CCT GAT ATC GAC AAT GTC ACG GTG CTG GAC TAT GAT GCC CGA Thr Val Pro Asp Ile Asp Asn Val Thr Val Leu Asp Tyr Asp Ala Arg 1615 1620 1625	5319
GAG CAG CGA GTT TAC TGG TCT GAT GTG CGG ACT CAA GCC ATC AAA AGG Glu Gln Arg Val Tyr Trp Ser Asp Val Arg Thr Gln Ala Ile Lys Arg 1630 1635 1640	5367
GCA TTT ATC AAC GGC ACT GGC GTG GAG ACC GTT GTC TCT GCA GAC TTG Ala Phe Ile Asn Gly Thr Gly Val Glu Thr Val Val Ser Ala Asp Leu 1645 1650 1655	5415
CCC AAC GCC CAC GGG CTG GCT GTG GAC TGG GTC TCC CGA AAT CTG TTT Pro Asn Ala His Gly Leu Ala Val Asp Trp Val Ser Arg Asn Leu Phe 1660 1665 1670	5463
TGG ACA AGT TAC GAC ACC AAC AAG AAG CAG ATT AAC GTG GCC CGG CTG Trp Thr Ser Tyr Asp Thr Asn Lys Lys Gln Ile Asn Val Ala Arg Leu 1675 1680 1685 1690	5511
GAC GGC TCC TTC AAG AAT GCG GTG GTG CAG GGC CTG GAG CAG CCC CAC Asp Gly Ser Phe Lys Asn Ala Val Val Gln Gly Leu Glu Gln Pro His 1695 1700 1705	5559
GGC CTG GTC GTC CAC CCG CTT CGT GGC AAG CTC TAC TGG ACT GAT GGG Gly Leu Val Val His Pro Leu Arg Gly Lys Leu Tyr Trp Thr Asp Gly 1710 1715 1720	5607

FIG.6A-10

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GAC AAC ATC AGC ATG GCC AAC ATG GAT GGG AGC AAC CAC ACT CTG CTC Asp Asn Ile Ser Met Ala Asn Met Asp Gly Ser Asn His Thr Leu Leu 1725 1730 1735	5655
TTC AGT GGC CAG AAG GGC CCT GTG GGG TTG GCC ATT GAC TTC CCT GAG Phe Ser Gly Gln Lys Gly Pro Val Gly Leu Ala Ile Asp Phe Pro Glu 1740 1745 1750	5703
AGC AAA CTC TAC TGG ATC AGC TCT GGG AAC CAC ACA ATC AAC CGT TGC Ser Lys Leu Tyr Trp Ile Ser Ser Gly Asn His Thr Ile Asn Arg Cys 1755 1760 1765 1770	5751
AAT CTG GAT GGG AGC GAG CTG GAG GTC ATC GAC ACC ATG CGG AGC CAG Asn Leu Asp Gly Ser Glu Leu Glu Val Ile Asp Thr Met Arg Ser Gln 1775 1780 1785	5799
CTG GGC AAG GCC ACT GCC CTG GCC ATC ATG GGG GAC AAG CTG TGG TGG Leu Gly Lys Ala Thr Ala Leu Ala Ile Met Gly Asp Lys Leu Trp Trp 1790 1795 1800	5847
GCA GAT CAG GTG TCA GAG AAG ATG GGC ACG TGC AAC AAA GCC GAT GGC Ala Asp Gln Val Ser Glu Lys Met Gly Thr Cys Asn Lys Ala Asp Gly 1805 1810 1815	5895
TCT GGG TCC GTG GTG CTG CGG AAC AGT ACC ACG TTG GTT ATG CAC ATG Ser Gly Ser Val Val Leu Arg Asn Ser Thr Thr Leu Val Met His Met 1820 1825 1830	5943
AAG GTG TAT GAC GAG AGC ATC CAG CTA GAG CAT GAG GGC ACC AAC CCC Lys Val Tyr Asp Glu Ser Ile Gln Leu Glu His Glu Gly Thr Asn Pro 1835 1840 1845 1850	5991
TGC AGT GTC AAC AAC GGA GAC TGT TCC CAG CTC TGC CTG CCA ACA TCA Cys Ser Val Asn Asn Gly Asp Cys Ser Gln Leu Cys Leu Pro Thr Ser 1855 1860 1865	6039
GAG ACG ACT CGC TCC TGT ATG TGT ACA GCC GGT TAC AGC CTC CGG AGC Glu Thr Thr Arg Ser Cys Met Cys Thr Ala Gly Tyr Ser Leu Arg Ser 1870 1875 1880	6087
GGA CAG CAG GCC TGT GAG GGT GTG GGC TCT TTT CTC CTG TAC TCT GTA Gly Gln Gln Ala Cys Glu Gly Val Gly Ser Phe Leu Leu Tyr Ser Val 1885 1890 1895	6135

FIG.6A-11

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CAT GAG GGA ATT CGG GGG ATT CCA CTA GAT CCC AAT GAC AAG TCG GAT His Glu Gly Ile Arg Gly Ile Pro Leu Asp Pro Asn Asp Lys Ser Asp 1900 1905 1910	6183
GCC CTG GTC CCA GTG TCC GGA ACT TCA CTG GCT GTC GGA ATC GAC TTC Ala Leu Val Pro Val Ser Gly Thr Ser Leu Ala Val Gly Ile Asp Phe 1915 1920 1925 1930	6231
CAT GCC GAA AAT GAC ACT ATT TAT TGG GTG GAT ATG GGC CTA AGC ACC His Ala Glu Asn Asp Thr Ile Tyr Trp Val Asp Met Gly Leu Ser Thr 1935 1940 1945	6279
ATC AGC AGG GCC AAG CGT GAC CAG ACA TGG CGA GAG GAT GTG GTG ACC Ile Ser Arg Ala Lys Arg Asp Gln Thr Trp Arg Glu Asp Val Val Thr 1950 1955 1960	6327
AAC GGT ATT GGC CGT GTG GAG GGC ATC GCC GTG GAC TGG ATC GCA GGC Asn Gly Ile Gly Arg Val Glu Gly Ile Ala Val Asp Trp Ile Ala Gly 1965 1970 1975	6375
AAC ATA TAC TGG ACG GAC CAG GGC TTC GAT GTC ATC GAG GTT GCC CGG Asn Ile Tyr Trp Thr Asp Gln Gly Phe Asp Val Ile Glu Val Ala Arg 1980 1985 1990	6423
CTC AAT GGC TCT TTT CGT TAT GTG GTC ATT TCC CAG GGT CTG GAC AAG Leu Asn Gly Ser Phe Arg Tyr Val Val Ile Ser Gln Gly Leu Asp Lys 1995 2000 2005 2010	6471
CCT CGG GCC ATC ACT GTC CAC CCA GAG AAG GGG TAC TTG TTC TGG ACC Pro Arg Ala Ile Thr Val His Pro Glu Lys Gly Tyr Leu Phe Trp Thr 2015 2020 2025	6519
GAG TGG GGT CAT TAC CCA CGT ATT GAG CGG TCT CGC CTT GAT GGC ACA Glu Trp Gly His Tyr Pro Arg Ile Glu Arg Ser Arg Leu Asp Gly Thr 2030 2035 2040	6567
GAG AGA GTG GTG TTG GTT AAT GTC AGC ATC AGC TGG CCC AAT GGC ATC Glu Arg Val Val Leu Val Asn Val Ser Ile Ser Trp Pro Asn Gly Ile 2045 2050 2055	6615
TCA GTA GAC TAT CAG GGC GGC AAG CTC TAC TGG TGT GAT GCT CGG ATG Ser Val Asp Tyr Gln Gly Gly Lys Leu Tyr Trp Cys Asp Ala Arg Met 2060 2065 2070	6663

FIG.6A-12

SUBSTITUTE SHEET (RULE 26)

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GAC Asp	AAG Lys	ATC Ile	GAG Glu	CGC Arg	ATC Ile	GAC Asp	CTG Leu	GAA Glu	ACG Thr	GGC Gly	GAG Glu	AAC Asn	CGG Arg	GAG Glu	GTG Val	6711
2075			2080			2085			2090							
GTC Val	CTG Leu	TCC Ser	AGC Ser	AAT Asn	AAC Asn	ATG Met	GAT Asp	ATG Met	TTC Phe	TCC Ser	GTG Val	TCC Ser	GTG Val	TTT Phe	GAG Glu	6759
			2095			2100						2105				
GAC Asp	TTC Phe	ATC Ile	TAC Tyr	TGG Trp	AGT Ser	GAC Asp	AGA Arg	ACT Thr	CAC His	GCC Ala	AAT Asn	GGC Gly	TCC Ser	ATC Ile	AAG Lys	6807
			2110			2115						2120				
CGC Arg	GGC Gly	TGC Cys	AAA Lys	GAC Asp	AAT Asn	GCT Ala	ACA Thr	GAC Asp	TCC Ser	GTG Val	CCT Pro	CTG Leu	AGG Arg	ACA Thr	GGC Gly	6855
2125						2130						2135				
ATT Ile	GGT Gly	GTT Val	CAG Gln	CTT Leu	AAA Lys	GAC Asp	ATC Ile	AAG Lys	GTC Val	TTC Phe	AAC Asn	AGG Arg	GAC Asp	AGG Arg	CAG Gln	6903
2140						2145						2150				
AAG Lys	GGT Gly	ACC Thr	AAT Asn	GTG Val	TGC Cys	GCG Ala	GTA Val	GCC Ala	AAC Asn	GGC Gly	GGG Gly	TGC Cys	CAG Gln	CAG Gln	CTC Leu	6951
2155			2160						2165			2170				
TGC Cys	TTG Leu	TAT Tyr	CGG Arg	GGT Gly	GGC Gly	GGA Gly	CAG Gln	CGA Arg	GCC Ala	TGT Cys	GCC Ala	TGT Cys	GCC Ala	CAC His	GGG Gly	6999
			2175						2180						2185	
ATG Met	CTG Leu	GCA Ala	GAA Glu	GAC Asp	GGG Gly	GCC Ala	TCA Ser	TGC Cys	CGA Arg	GAG Glu	TAC Tyr	GCT Ala	GGC Gly	TAC Tyr	CTG Leu	7047
			2190			2195						2200				
CTC Leu	TAC Tyr	TCA Ser	GAG Glu	CGG Arg	ACC Thr	ATC Ile	CTC Leu	AAG Lys	AGC Ser	ATC Ile	CAC His	CTG Leu	TCG Ser	GAT Asp	GAG Glu	7095
2205						2210						2215				
CGT Arg	AAC Asn	CTC Leu	AAC Asn	GCA Ala	CCG Pro	GTG Val	CAG Gln	CCC Pro	TTT Phe	GAA Glu	GAC Asp	CCC Pro	GAG Glu	CAC His	ATG Met	7143
2220			2225						2230							
AAA Lys	AAT Asn	GTC Val	ATC Ile	GCC Ala	CTG Leu	GCC Ala	TTT Phe	GAC Asp	TAC Tyr	CGA Arg	GCA Ala	GGC Gly	ACC Thr	TCC Ser	CCG Pro	7191
2235			2240						2245			2250				

FIG. 6A-13

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GGG ACC CCT AAC CGC ATC TTC TTC AGT GAC ATC CAC TTT GGG AAC ATC Gly Thr Pro Asn Arg Ile Phe Phe Ser Asp Ile His Phe Gly Asn Ile 2255 2260 2265	7239
CAG CAG ATC AAT GAC GAT GGC TCG GGC AGG ACC ACC ATC GTG GAA AAT Gln Gln Ile Asn Asp Asp Gly Ser Gly Arg Thr Thr Ile Val Glu Asn 2270 2275 2280	7287
GTG GGC TCT GTG GAA GGC CTG GCC TAT CAC CGT GGC TGG GAC ACA CTG Val Gly Ser Val Glu Gly Leu Ala Tyr His Arg Gly Trp Asp Thr Leu 2285 2290 2295	7335
TAC TGG ACA AGC TAC ACC ACA TCC ACC ATC ACC CGC CAC ACC GTG GAC Tyr Trp Thr Ser Tyr Thr Thr Ser Thr Ile Thr Arg His Thr Val Asp 2300 2305 2310	7383
CAG ACT CGC CCA GGG GCC TTC GAG AGG GAG ACA GTC ATC ACC ATG TCC Gln Thr Arg Pro Gly Ala Phe Glu Arg Glu Thr Val Ile Thr Met Ser 2315 2320 2325 2330	7431
GGA GAC GAC CAC CCG AGA GCC TTT GTG CTG GAT GAG TGC CAG AAC CTG Gly Asp Asp His Pro Arg Ala Phe Val Leu Asp Glu Cys Gln Asn Leu 2335 2340 2345	7479
ATG TTC TGG ACC AAT TGG AAC GAG CTC CAT CCA AGC ATC ATG CGG GCA Met Phe Trp Thr Asn Trp Asn Glu Leu His Pro Ser Ile Met Arg Ala 2350 2355 2360	7527
GCC CTA TCC GGA GCC AAC GTC CTG ACC CTC ATT GAG AAG GAC ATC CGC Ala Leu Ser Gly Ala Asn Val Leu Thr Leu Ile Glu Lys Asp Ile Arg 2365 2370 2375	7575
ACG CCC AAT GGG TTG GCC ATC GAC CAC CGG GCG GAG AAG CTG TAC TTC Thr Pro Asn Gly Leu Ala Ile Asp His Arg Ala Glu Lys Leu Tyr Phe 2380 2385 2390	7623
TCG GAT GCC ACC TTG GAC AAG ATC GAG CGC TGC GAG TAC GAC GGC TCC Ser Asp Ala Thr Leu Asp Lys Ile Glu Arg Cys Glu Tyr Asp Gly Ser 2395 2400 2405 2410	7671
CAC CGC TAT GTG ATC CTA AAG TCG GAG CCC GTC CAC CCC TTT GGG TTG His Arg Tyr Val Ile Leu Lys Ser Glu Pro Val His Pro Phe Gly Leu 2415 2420 2425	7719

FIG.6A-14

SUBSTITUTE SHEET (RULE 26)

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GCG GTG TAC GGA GAG CAC ATT TTC TGG ACT GAC TGG GTG CGG CGG GCT Ala Val Tyr Gly Glu His Ile Phe Trp Thr Asp Trp Val Arg Arg Ala 2430 2435 2440	7767
GTG CAG CGA GCC AAC AAG TAT GTG GGC AGC GAC ATG AAG CTG CTT CGG Val Gln Arg Ala Asn Lys Tyr Val Gly Ser Asp Met Lys Leu Leu Arg 2445 2450 2455	7815
GTG GAC ATT CCC CAG CAA CCC ATG GGC ATC ATC GCC GTG GCC AAT GAC Val Asp Ile Pro Gln Gln Pro Met Gly Ile Ile Ala Val Ala Asn Asp 2460 2465 2470	7863
ACC AAC AGC TGT GAA CTC TCC CCC TGC CGT ATC AAC AAT GGA GGC TGC Thr Asn Ser Cys Glu Leu Ser Pro Cys Arg Ile Asn Asn Gly Gly Cys 2475 2480 2485 2490	7911
CAG GAT CTG TGT CTG CTC ACC CAC CAA GGC CAC GTC AAC TGT TCC TGT Gln Asp Leu Cys Leu Leu Thr His Gln Gly His Val Asn Cys Ser Cys 2495 2500 2505	7959
CGA GGG GGC CGG ATC CTC CAG GAG GAC TTC ACC TGC CGG GCT GTG AAC Arg Gly Gly Arg Ile Leu Gln Glu Asp Phe Thr Cys Arg Ala Val Asn 2510 2515 2520	8007
TCC TCT TGT CGG GCA CAA GAT GAG TTT GAG TGT GCC AAT GGG GAA TGT Ser Ser Cys Arg Ala Gln Asp Glu Phe Glu Cys Ala Asn Gly Glu Cys 2525 2530 2535	8055
ATC AGC TTC AGC CTC ACC TGT GAT GGC GTC TCC CAC TGC AAG GAC AAG Ile Ser Phe Ser Leu Thr Cys Asp Gly Val Ser His Cys Lys Asp Lys 2540 2545 2550	8103
TCC GAT GAG AAG CCC TCC TAC TGC AAC TCA CGC CGC TGC AAG AAG ACT Ser Asp Glu Lys Pro Ser Tyr Cys Asn Ser Arg Arg Cys Lys Lys Thr 2555 2560 2565 2570	8151
TTC CGC CAG TGT AAC AAT GGC CGC TGT GTA TCC AAC ATG CTG TGG TGC Phe Arg Gln Cys Asn Asn Gly Arg Cys Val Ser Asn Met Leu Trp Cys 2575 2580 2585	8199
AAT GGG GTG GAT TAC TGT GGG GAT GGC TCT GAT GAG ATA CCT TGC AAC Asn Gly Val Asp Tyr Cys Gly Asp Gly Ser Asp Glu Ile Pro Cys Asn 2590 2595 2600	8247

FIG.6A-15

SUBSTITUTE SHEET (RULE 26)

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AAG ACT GCC TGT GGT GTG GGT GAG TTC CGC TGC CGG GAT GGG TCC TGC Lys Thr Ala Cys Gly Val Gly Glu Phe Arg Cys Arg Asp Gly Ser Cys 2605 2610 2615	8295
ATC GGG AAC TCC AGT CGC TGC AAC CAG TTT GTG GAT TGT GAG GAT GCC Ile Gly Asn Ser Ser Arg Cys Asn Gln Phe Val Asp Cys Glu Asp Ala 2620 2625 2630	8343
TCG GAT GAG ATG AAT TGC AGT GCC ACA GAC TGC AGC AGC TAT TTC CGC Ser Asp Glu Met Asn Cys Ser Ala Thr Asp Cys Ser Ser Tyr Phe Arg 2635 2640 2645 2650	8391
CTG GGC GTG AAA GGT GTC CTC TTC CAG CCG TGC GAG CGG ACA TCC CTG Leu Gly Val Lys Gly Val Leu Phe Gln Pro Cys Glu Arg Thr Ser Leu 2655 2660 2665	8439
TGC TAC GCA CCT AGC TGG GTG TGT GAT GGC GCC AAC GAC TGT GGA GAC Cys Tyr Ala Pro Ser Trp Val Cys Asp Gly Ala Asn Asp Cys Gly Asp 2670 2675 2680	8487
TAC AGC GAT GAA CGT GAC TGT CCA GGT GTG AAG CGC CCT AGG TGC CCG Tyr Ser Asp Glu Arg Asp Cys Pro Gly Val Lys Arg Pro Arg Cys Pro 2685 2690 2695	8535
CTC AAT TAC TTT GCC TGC CCC AGC GGG CGC TGT ATC CCC ATG AGC TGG Leu Asn Tyr Phe Ala Cys Pro Ser Gly Arg Cys Ile Pro Met Ser Trp 2700 2705 2710	8583
ACG TGT GAC AAG GAG GAT GAC TGT GAG AAC GGC GAG GAT GAG ACC CAC Thr Cys Asp Lys Glu Asp Asp Cys Glu Asn Gly Glu Asp Glu Thr His 2715 2720 2725 2730	8631
TGC AAC AAG TTC TGC TCA GAG GCA CAG TTC GAG TGC CAG AAC CAC CGG Cys Asn Lys Phe Cys Ser Glu Ala Gln Phe Glu Cys Gln Asn His Arg 2735 2740 2745	8679
TGT ATC TCC AAG CAG TGG CTG TGT GAC GGT AGC GAT GAT TGC GGG GAT Cys Ile Ser Lys Gln Trp Leu Cys Asp Gly Ser Asp Asp Cys Gly Asp 2750 2755 2760	8727
GGC TCC GAT GAG GCA GCT CAC TGT GAA GGC AAG ACA TGT GGC CCC TCC Gly Ser Asp Glu Ala Ala His Cys Glu Gly Lys Thr Cys Gly Pro Ser 2765 2770 2775	8775

FIG.6A-16

SUBSTITUTE SHEET (RULE 26)

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TCC TTC TCC TGT CCC GGC ACC CAC GTG TGT GTC CCT GAG CGC TGG CTC	8823
Ser Phe Ser Cys Pro Gly Thr His Val Cys Val Pro Glu Arg Trp Leu	
2780 2785 2790	
TGT GAT GGC GAC AAG GAC TGT ACC GAT GGC GCG GAT GAG AGT GTC ACT	8871
Cys Asp Gly Asp Lys Asp Cys Thr Asp Gly Ala Asp Glu Ser Val Thr	
2795 2800 2805 2810	
GCT GGC TGC CTG TAC AAC AGC ACC TGT GAT GAC CGT GAG TTC ATG TGC	8919
Ala Gly Cys Leu Tyr Asn Ser Thr Cys Asp Asp Arg Glu Phe Met Cys	
2815 2820 2825	
CAG AAC CGC TTG TGT ATT CCC AAG CAT TTC GTG TGC GAC CAT GAC CGT	8967
Gln Asn Arg Leu Cys Ile Pro Lys His Phe Val Cys Asp His Asp Arg	
2830 2835 2840	
GAC TGT GCT GAT GGC TCT GAT GAA TCC CCT GAG TGT GAG TAC CCA ACC	9015
Asp Cys Ala Asp Gly Ser Asp Glu Ser Pro Glu Cys Glu Tyr Pro Thr	
2845 2850 2855	
TGC GGG CCC AAT GAA TTC CGC TGT GCC AAT GGG CGT TGT CTG AGC TCC	9063
Cys Gly Pro Asn Glu Phe Arg Cys Ala Asn Gly Arg Cys Leu Ser Ser	
2860 2865 2870	
CGT CAG TGG GAA TGT GAT GGG GAG AAT GAC TGT CAC GAC CAC AGC GAT	9111
Arg Gln Trp Glu Cys Asp Gly Glu Asn Asp Cys His Asp His Ser Asp	
2875 2880 2885 2890	
GAG GCT CCC AAG AAC CCA CAC TGC ACC AGC CCA GAG CAC AAA TGC AAT	9159
Glu Ala Pro Lys Asn Pro His Cys Thr Ser Pro Glu His Lys Cys Asn	
2895 2900 2905	
GCC TCA TCA CAG TTC CTG TGC AGC AGC GGG CGC TGC GTG GCT GAG GCG	9207
Ala Ser Ser Gln Phe Leu Cys Ser Ser Gly Arg Cys Val Ala Glu Ala	
2910 2915 2920	
TTG CTC TGC AAC GGC CAG GAC GAC TGT GGG GAC GGT TCA GAC GAA CGC	9255
Leu Leu Cys Asn Gly Gln Asp Asp Cys Gly Asp Gly Ser Asp Glu Arg	
2925 2930 2935	
GGG TGC CAT GTC AAC GAG TGT CTC AGC CGC AAG CTC AGT GGC TGC AGT	9303
Gly Cys His Val Asn Glu Cys Leu Ser Arg Lys Leu Ser Gly Cys Ser	
2940 2945 2950	

FIG.6A-17

SUBSTITUTE SHEET (RULE 26)

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CAG GAC TGC GAG GAC CTC AAG ATA GGC TTT AAG TGC CGC TGT CGC CCG Gln Asp Cys Glu Asp Leu Lys Ile Gly Phe Lys Cys Arg Cys Arg Pro 2955 2960 2965 2970	9351
GGC TTC CGG CTA AAG GAC GAT GGC AGG ACC TGT GCC GAC CTG GAT GAG Gly Phe Arg Leu Lys Asp Asp Gly Arg Thr Cys Ala Asp Leu Asp Glu 2975 2980 2985	9399
TGC AGC ACC ACC TTC CCC TGC AGC CAG CTC TGC ATC AAC ACC CAC GGA Cys Ser Thr Thr Phe Pro Cys Ser Gln Leu Cys Ile Asn Thr His Gly 2990 2995 3000	9447
AGT TAC AAG TGT CTG TGT GTG GAG GGC TAT GCA CCC CGT GGC GGT GAC Ser Tyr Lys Cys Leu Cys Val Glu Gly Tyr Ala Pro Arg Gly Gly Asp 3005 3010 3015	9495
CCC CAC AGC TGC AAA GCT GTG ACC GAT GAG GAG CCA TTT CTC ATC TTT Pro His Ser Cys Lys Ala Val Thr Asp Glu Glu Pro Phe Leu Ile Phe 3020 3025 3030	9543
GCC AAC CGG TAC TAC CTG CGG AAG CTC AAC CTG GAC GGC TCC AAC TAC Ala Asn Arg Tyr Tyr Leu Arg Lys Leu Asn Leu Asp Gly Ser Asn Tyr 3035 3040 3045 3050	9591
ACA CTG CTT AAG CAG GGC CTG AAC AAT GCG GTC GCC TTG GCA TTT GAC Thr Leu Leu Lys Gln Gly Leu Asn Asn Ala Val Ala Leu Ala Phe Asp 3055 3060 3065	9639
TAC CGA GAG CAG ATG ATC TAC TGG ACG GGC GTG ACC ACC CAG GGC AGC Tyr Arg Glu Gln Met Ile Tyr Trp Thr Gly Val Thr Thr Gln Gly Ser 3070 3075 3080	9687
ATG ATT CGC AGG ATG CAC CTC AAC GGC AGC AAC GTG CAG GTT CTG CAC Met Ile Arg Arg Met His Leu Asn Gly Ser Asn Val Gln Val Leu His 3085 3090 3095	9735
CGG ACG GGC CTT AGT AAC CCA GAT GGG CTC GCT GTG GAC TGG GTG GGT Arg Thr Gly Leu Ser Asn Pro Asp Gly Leu Ala Val Asp Trp Val Gly 3100 3105 3110	9783
GGC AAC CTG TAC TGG TGT GAC AAG GGC AGA GAT ACC ATT GAG GTG TCC Gly Asn Leu Tyr Trp Cys Asp Lys Gly Arg Asp Thr Ile Glu Val Ser 3115 3120 3125 3130	9831

FIG. 6A-18

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AAG CTT AAC GGG GCC TAT CGG ACA GTG CTG GTC AGC TCT GGC CTC CGG Lys Leu Asn Gly Ala Tyr Arg Thr Val Leu Val Ser Ser Gly Leu Arg 3135 3140 3145	9879
GAG CCC AGA GCT CTG GTA GTG GAT GTA CAG AAT GGG TAC CTG TAC TGG Glu Pro Arg Ala Leu Val Val Asp Val Gln Asn Gly Tyr Leu Tyr Trp 3150 3155 3160	9927
ACA GAC TGG GGT GAC CAC TCA CTG ATC GGC CGG ATT GGC ATG GAT GGA Thr Asp Trp Gly Asp His Ser Leu Ile Gly Arg Ile Gly Met Asp Gly 3165 3170 3175	9975
TCT GGC CGC AGC ATC ATC GTG GAC ACT AAG ATC ACA TGG CCC AAT GGC Ser Gly Arg Ser Ile Ile Val Asp Thr Lys Ile Thr Trp Pro Asn Gly 3180 3185 3190	10023
CTG ACC GTG GAC TAC GTC ACG GAA CGC ATC TAC TGG GCT GAC GCC CGT Leu Thr Val Asp Tyr Val Thr Glu Arg Ile Tyr Trp Ala Asp Ala Arg 3195 3200 3205 3210	10071
GAG GAC TAC ATC GAG TTC GCC AGC CTG GAT GGC TCC AAC CGT CAC GTT Glu Asp Tyr Ile Glu Phe Ala Ser Leu Asp Gly Ser Asn Arg His Val 3215 3220 3225	10119
GTG CTG AGC CAA GAC ATC CCA CAC ATC TTT GCG CTG ACC CTA TTT GAA Val Leu Ser Gln Asp Ile Pro His Ile Phe Ala Leu Thr Leu Phe Glu 3230 3235 3240	10167
GAC TAC GTC TAC TGG ACA GAC TGG GAA ACG AAG TCC ATC AAC CGG GCC Asp Tyr Val Tyr Trp Thr Asp Trp Glu Thr Lys Ser Ile Asn Arg Ala 3245 3250 3255	10215
CAC AAG ACC ACG GGT GCC AAC AAA ACA CTC CTC ATC AGC ACC CTG CAC His Lys Thr Thr Gly Ala Asn Lys Thr Leu Leu Ile Ser Thr Leu His 3260 3265 3270	10263
CGG CCC ATG GAC TTA CAT GTA TTC CAC GCC CTG CGC CAG CCA GAT GTG Arg Pro Met Asp Leu His Val Phe His Ala Leu Arg Gln Pro Asp Val 3275 3280 3285 3290	10311
CCC AAT CAC CCC TGC AAA GTC AAC AAT GGT GGC TGC AGC AAC CTG TGC Pro Asn His Pro Cys Lys Val Asn Asn Gly Gly Cys Ser Asn Leu Cys 3295 3300 3305	10359

FIG.6A-19

SUBSTITUTE SHEET (RULE 26)

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CTG CTG TCC CCT GGG GGT GGT CAC AAG TGC GCC TGC CCC ACC AAC TTC 10407
 Leu Leu Ser Pro Gly Gly Gly His Lys Cys Ala Cys Pro Thr Asn Phe
 3310 3315 3320

TAT CTG GGT GGC GAT GGC CGT ACC TGT GTG TCC AAC TGC ACA GCA AGC 10455
 Tyr Leu Gly Gly Asp Gly Arg Thr Cys Val Ser Asn Cys Thr Ala Ser
 3325 3330 3335

CAG TTT GTG TGC AAA AAT GAC AAG TGC ATC CCC TTC TGG TGG AAG TGT 10503
 Gln Phe Val Cys Lys Asn Asp Lys Cys Ile Pro Phe Trp Trp Lys Cys
 3340 3345 3350

GAC ACG GAG GAC GAC TGT GGG GAT CAC TCA GAC GAG CCT CCA GAC TGT 10551
 Asp Thr Glu Asp Asp Cys Gly Asp His Ser Asp Glu Pro Pro Asp Cys
 3355 3360 3365 3370

CCC GAG TTC AAG TGC CGC CCA GGC CAG TTC CAG TGC TCC ACC GGC ATC 10599
 Pro Glu Phe Lys Cys Arg Pro Gly Gln Phe Gln Cys Ser Thr Gly Ile
 3375 3380 3385

TGC ACC AAC CCT GCC TTC ATC TGT GAT GGG GAC AAT GAC TGC CAA GAC 10647
 Cys Thr Asn Pro Ala Phe Ile Cys Asp Gly Asp Asn Asp Cys Gln Asp
 3390 3395 3400

AAT AGT GAC GAG GCC AAT TGC GAC ATT CAC GTC TGC TTG CCC AGC CAA 10695
 Asn Ser Asp Glu Ala Asn Cys Asp Ile His Val Cys Leu Pro Ser Gln
 3405 3410 3415

TTC AAG TGC ACC AAC ACC AAC CGC TGC ATT CCT GGC ATC TTC CGT TGC 10743
 Phe Lys Cys Thr Asn Thr Asn Arg Cys Ile Pro Gly Ile Phe Arg Cys
 3420 3425 3430

AAT GGG CAG GAC AAC TGC GGG GAC GGC GAG GAT GAG CGG GAT TGC CCT 10791
 Asn Gly Gln Asp Asn Cys Gly Asp Gly Glu Asp Glu Arg Asp Cys Pro
 3435 3440 3445 3450

GAG GTG ACC TGC GCC CCC AAC CAG TTC CAG TGC TCC ATC ACC AAG CGC 10839
 Glu Val Thr Cys Ala Pro Asn Gln Phe Gln Cys Ser Ile Thr Lys Arg
 3455 3460 3465

TGC ATC CCT CGC GTC TGG GTC TGT GAC AGG GAT AAT CAC TGT GTG GAC 10887
 Cys Ile Pro Arg Val Trp Val Cys Asp Arg Asp Asn His Cys Val Asp
 3470 3475 3480

FIG.6A-20

SUBSTITUTE SHEET (RULE 26)

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GGC AGT GAT GAG CCT GCC AAC TGT ACC CAA ATG ACC TGT GGA GTG GAT Gly Ser Asp Glu Pro Ala Asn Cys Thr Gln Met Thr Cys Gly Val Asp 3485 3490 3495	10935
GAG TTC CGC TGC AAG GAT TCT GGC CGC TGC ATC CCC GCG CGC TGG AAG Glu Phe Arg Cys Lys Asp Ser Gly Arg Cys Ile Pro Ala Arg Trp Lys 3500 3505 3510	10983
TGT GAC GGA GAA GAT GAC TGT GGG GAT GGT TCA GAT GAG CCC AAG GAA Cys Asp Gly Glu Asp Asp Cys Gly Asp Gly Ser Asp Glu Pro Lys Glu 3515 3520 3525 3530	11031
GAG TGT GAT GAG CGC ACC TGT GAG CCA TAC CAG TTC CGC TGC AAA AAC Glu Cys Asp Glu Arg Thr Cys Glu Pro Tyr Gln Phe Arg Cys Lys Asn 3535 3540 3545	11079
AAC CGC TGT GTC CCA GGC CGT TGG CAA TGT GAC TAC GAC AAC GAC TGC Asn Arg Cys Val Pro Gly Arg Trp Gln Cys Asp Tyr Asp Asn Asp Cys 3550 3555 3560	11127
GGA GAT AAC TCG GAC GAG GAG AGC TGC ACA CCT CGG CCC TGC TCT GAG Gly Asp Asn Ser Asp Glu Glu Ser Cys Thr Pro Arg Pro Cys Ser Glu 3565 3570 3575	11175
AGT GAG TTT TTC TGT GCC AAT GGC CGC TGC ATC GCT GGG CGC TGG AAG Ser Glu Phe Phe Cys Ala Asn Gly Arg Cys Ile Ala Gly Arg Trp Lys 3580 3585 3590	11223
TGT GAT GGG GAC CAT GAC TGT GCC GAC GGC TCA GAC GAG AAA GAC TGC Cys Asp Gly Asp His Asp Cys Ala Asp Gly Ser Asp Glu Lys Asp Cys 3595 3600 3605 3610	11271
ACC CCC CGC TGT GAT ATG GAC CAG TTC CAG TGC AAG AGT GGC CAC TGC Thr Pro Arg Cys Asp Met Asp Gln Phe Gln Cys Lys Ser Gly His Cys 3615 3620 3625	11319
ATC CCC CTG CGC TGG CCG TGT GAC GCG GAT GCT GAC TGT ATG GAC GGC Ile Pro Leu Arg Trp Pro Cys Asp Ala Asp Ala Asp Cys Met Asp Gly 3630 3635 3640	11367
AGT GAC GAG GAA GCC TGT GGC ACT GGG GTG AGG ACC TGC CCA TTG GAT Ser Asp Glu Glu Ala Cys Gly Thr Gly Val Arg Thr Cys Pro Leu Asp 3645 3650 3655	11415

FIG.6A-21

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GAG TTT CAA TGT AAC AAC ACC TGT TGC AAG CCG CTG GCC TGG AAG TGT Glu Phe Gln Cys Asn Asn Thr Leu Cys Lys Pro Leu Ala Trp Lys Cys 3660 3665 3670	11463
GAT GGA GAG GAC GAC TGT GGG GAC AAC TCA GAT GAG AAC CCC GAG GAA Asp Gly Glu Asp Asp Cys Gly Asp Asn Ser Asp Glu Asn Pro Glu Glu 3675 3680 3685 3690	11511
TGC GCC CGG TTC ATC TGC CCT CCC AAC CGG CCT TTC CGC TGC AAG AAT Cys Ala Arg Phe Ile Cys Pro Pro Asn Arg Pro Phe Arg Cys Lys Asn 3695 3700 3705	11559
GAC CGA GTC TGC CTG TGG ATT GGG CGC CAG TGT GAT GGC GTG GAC AAC Asp Arg Val Cys Leu Trp Ile Gly Arg Gln Cys Asp Gly Val Asp Asn 3710 3715 3720	11607
TGT GGA GAT GGG ACT GAC GAG GAG GAC TGT GAG CCC CCC ACG GCC CAG Cys Gly Asp Gly Thr Asp Glu Glu Asp Cys Glu Pro Pro Thr Ala Gln 3725 3730 3735	11655
AAC CCC CAC TGC AAA GAC AAG AAG GAG TTC CTG TGC CGA AAC CAG CGC Asn Pro His Cys Lys Asp Lys Lys Glu Phe Leu Cys Arg Asn Gln Arg 3740 3745 3750	11703
TGT CTA TCA TCC TCC CTG CGC TGT AAC ATG TTC GAT GAC TGC GGC GAT Cys Leu Ser Ser Ser Leu Arg Cys Asn Met Phe Asp Asp Cys Gly Asp 3755 3760 3765 3770	11751
GGC TCC GAT GAA GAA GAT TGC AGC ATC GAC CCC AAG CTG ACC AGC TGT Gly Ser Asp Glu Glu Asp Cys Ser Ile Asp Pro Lys Leu Thr Ser Cys 3775 3780 3785	11799
GCC ACC AAT GCC AGC ATG TGT GGG GAC GAA GCT CGT TGT GTG CGC ACT Ala Thr Asn Ala Ser Met Cys Gly Asp Glu Ala Arg Cys Val Arg Thr 3790 3795 3800	11847
GAG AAA GCT GCC TAC TGT GCC TGC CGC TCG GGC TTC CAT ACT GTG CCG Glu Lys Ala Ala Tyr Cys Ala Cys Arg Ser Gly Phe His Thr Val Pro 3805 3810 3815	11895
GGC CAG CCC GGA TGC CAG GAC ATC AAC GAG TGC CTG CGC TTT GGT ACC Gly Gln Pro Gly Cys Gln Asp Ile Asn Glu Cys Leu Arg Phe Gly Thr 3820 3825 3830	11943

FIG.6A-22

SUBSTITUTE SHEET (RULE 26)

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TGC TCT CAG CTC TGG AAC AAA CCC AAG GGA GGC CAC CTC TGC AGC TGT	11991
Cys Ser Gln Leu Trp Asn Lys Pro Lys Gly Gly His Leu Cys Ser Cys	
3835 3840 3845 3850	
GCC CGC AAC TTC ATG AAG ACA CAC AAC ACC TGC AAA GCT GAA GGC TCC	12039
Ala Arg Asn Phe Met Lys Thr His Asn Thr Cys Lys Ala Glu Gly Ser	
3855 3860 3865	
GAG TAC CAG GTG CTA TAC ATC GCG GAT GAC AAC GAG ATC CGC AGC TTG	12087
Glu Tyr Gln Val Leu Tyr Ile Ala Asp Asp Asn Glu Ile Arg Ser Leu	
3870 3875 3880	
TTC CCG GGC CAC CCC CAC TCA GCC TAC GAG CAG ACA TTC CAG GGC GAT	12135
Phe Pro Gly His Pro His Ser Ala Tyr Glu Gln Thr Phe Gln Gly Asp	
3885 3890 3895	
GAG AGT GTC CGC ATA GAT GCC ATG GAT GTC CAT GTC AAG GCC GGC CGT	12183
Glu Ser Val Arg Ile Asp Ala Met Asp Val His Val Lys Ala Gly Arg	
3900 3905 3910	
GTC TAC TGG ACT AAC TGG CAC ACG GGC ACA ATC TCC TAC AGG AGC CTG	12231
Val Tyr Trp Thr Asn Trp His Thr Gly Thr Ile Ser Tyr Arg Ser Leu	
3915 3920 3925 3930	
CCC CCT GCC GCC CCT CCT ACC ACT TCC AAC CGC CAC CGG AGG CAG ATC	12279
Pro Pro Ala Ala Pro Pro Thr Thr Ser Asn Arg His Arg Arg Gln Ile	
3935 3940 3945	
GAC CGG GGT GTC ACC CAC CTC AAT ATT TCA GGG CTG AAG ATG CCG AGG	12327
Asp Arg Gly Val Thr His Leu Asn Ile Ser Gly Leu Lys Met Pro Arg	
3950 3955 3960	
GGT ATC GCT ATC GAC TGG GTG GCC GGG AAT GTG TAC TGG ACC GAT TCC	12375
Gly Ile Ala Ile Asp Trp Val Ala Gly Asn Val Tyr Trp Thr Asp Ser	
3965 3970 3975	
GGC CGA GAC GTG ATT GAG GTG GCG CAA ATG AAG GGC GAG AAC CGC AAG	12423
Gly Arg Asp Val Ile Glu Val Ala Gln Met Lys Gly Glu Asn Arg Lys	
3980 3985 3990	
ACG CTC ATC TCG GGC ATG ATT GAT GAG CCC CAT GCC ATC GTG GTG GAC	12471
Thr Leu Ile Ser Gly Met Ile Asp Glu Pro His Ala Ile Val Val Asp	
3995 4000 4005 4010	

FIG. 6A-23

34/65

CCT CTG AGG GGC ACC ATG TAC TGG TCA GAC TGG GGG AAC CAC CCC AAG	12519
Pro Leu Arg Gly Thr Met Tyr Trp Ser Asp Trp Gly Asn His Pro Lys	
4015 4020 4025	
ATT GAA ACA GCA GCG ATG GAT GGC ACC CTT CGG GAG ACT CTC GTG CAA	12567
Ile Glu Thr Ala Ala Met Asp Gly Thr Leu Arg Glu Thr Leu Val Gln	
4030 4035 4040	
GAC AAC ATT CAG TGG CCT ACA GGG CTG GCT GTG GAC TAT CAC AAT GAA	12615
Asp Asn Ile Gln Trp Pro Thr Gly Leu Ala Val Asp Tyr His Asn Glu	
4045 4050 4055	
CGG CTC TAC TGG GCA GAT GCC AAG CTT TCG GTC ATC GGC AGC ATC CGG	12663
Arg Leu Tyr Trp Ala Asp Ala Lys Leu Ser Val Ile Gly Ser Ile Arg	
4060 4065 4070	
CTC AAC GGC ACT GAC CCC ATT GTG GCT GCT GAC AGC AAA CGA GGC CTA	12711
Leu Asn Gly Thr Asp Pro Ile Val Ala Ala Asp Ser Lys Arg Gly Leu	
4075 4080 4085 4090	
AGT CAC CCC TTC AGC ATC GAT GTG TTT GAA GAC TAC ATC TAC GGA GTC	12759
Ser His Pro Phe Ser Ile Asp Val Phe Glu Asp Tyr Ile Tyr Gly Val	
4095 4100 4105	
ACT TAC ATC AAT AAT CGT GTC TTC AAG ATC CAC AAG TTT GGA CAC AGC	12807
Thr Tyr Ile Asn Asn Arg Val Phe Lys Ile His Lys Phe Gly His Ser	
4110 4115 4120	
CCC TTG TAC AAC CTA ACT GGG GGC CTG AGC CAT GCC TCT GAT GTA GTC	12855
Pro Leu Tyr Asn Leu Thr Gly Gly Leu Ser His Ala Ser Asp Val Val	
4125 4130 4135	
CTT TAC CAT CAA CAC AAG CAG CCT GAA GTG ACC AAC CCC TGT GAC CGC	12903
Leu Tyr His Gln His Lys Gln Pro Glu Val Thr Asn Pro Cys Asp Arg	
4140 4145 4150	
AAG AAA TGC GAA TGG CTG TGT CTG CTG AGC CCC AGC GGG CCT GTC TGC	12951
Lys Lys Cys Glu Trp Leu Cys Leu Leu Ser Pro Ser Gly Pro Val Cys	
4155 4160 4165 4170	
ACC TGT CCC AAT GGA AAG AGG CTG GAT AAT GGC ACC TGT GTG CCT GTG	12999
Thr Cys Pro Asn Gly Lys Arg Leu Asp Asn Gly Thr Cys Val Pro Val	
4175 4180 4185	

FIG.6A-24

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CCC TCT CCA ACA CCC CCT CCA GAT GCC CCT AGG CCT GGA ACC TGC ACT	13047
Pro Ser Pro Thr Pro Pro Pro Asp Ala Pro Arg Pro Gly Thr Cys Thr	
4190 4195 4200	
CTG CAG TGC TTC AAT GGT GGT AGT TGT TTC CTC AAC GCT CGG AGG CAG	13095
Leu Gln Cys Phe Asn Gly Gly Ser Cys Phe Leu Asn Ala Arg Arg Gln	
4205 4210 4215	
CCC AAG TGC CGT TGC CAG CCC CGT TAC ACA GGC GAT AAG TGT GAG CTG	13143
Pro Lys Cys Arg Cys Gln Pro Arg Tyr Thr Gly Asp Lys Cys Glu Leu	
4220 4225 4230	
GAT CAG TGC TGG GAA TAC TGT CAC AAC GGA GGC ACC TGT GCG GCT TCC	13191
Asp Gln Cys Trp Glu Tyr Cys His Asn Gly Gly Thr Cys Ala Ala Ser	
4235 4240 4245 4250	
CCA TCT GGC ATG CCC ACG TGC CGC TGT CCC ACT GGC TTC ACG GGC CCC	13239
Pro Ser Gly Met Pro Thr Cys Arg Cys Pro Thr Gly Phe Thr Gly Pro	
4255 4260 4265	
AAA TGC ACC GCA CAG GTG TGT GCA GGC TAC TGC TCT AAC AAC AGC ACC	13287
Lys Cys Thr Ala Gln Val Cys Ala Gly Tyr Cys Ser Asn Asn Ser Thr	
4270 4275 4280	
TGC ACC GTC AAC CAG GGC AAC CAG CCC CAG TGC CGA TGT CTA CCT GGC	13335
Cys Thr Val Asn Gln Gly Asn Gln Pro Gln Cys Arg Cys Leu Pro Gly	
4285 4290 4295	
TTC CTG GGC GAC CGT TGC CAG TAC CGG CAG TGC TCT GGC TTC TGT GAG	13383
Phe Leu Gly Asp Arg Cys Gln Tyr Arg Gln Cys Ser Gly Phe Cys Glu	
4300 4305 4310	
AAC TTT GGC ACC TGT CAG ATG GCT GCT GAT GGC TCC CGA CAA TGT CGC	13431
Asn Phe Gly Thr Cys Gln Met Ala Ala Asp Gly Ser Arg Gln Cys Arg	
4315 4320 4325 4330	
TGC ACC GTC TAC TTT GAG GGA CCA AGG TGT GAG GTG AAC AAG TGT AGT	13479
Cys Thr Val Tyr Phe Glu Gly Pro Arg Cys Glu Val Asn Lys Cys Ser	
4335 4340 4345	
CGC TGT CTC CAA GGC GCC TGT GTG GTC AAT AAG CAG ACC GGA GAT GTC	13527
Arg Cys Leu Gln Gly Ala Cys Val Val Asn Lys Gln Thr Gly Asp Val	
4350 4355 4360	

FIG.6A-25

36/65

ACA TGC AAC TGC ACT GAT GGC CGG GTA GCC CCC AGT TGT CTC ACC TGC 13575
 Thr Cys Asn Cys Thr Asp Gly Arg Val Ala Pro Ser Cys Leu Thr Cys
 4365 4370 4375

ATC GAT CAC TGT AGC AAT GGT GGC TCC TGC ACC ATG AAC AGC AAG ATG 13623
 Ile Asp His Cys Ser Asn Gly Gly Ser Cys Thr Met Asn Ser Lys Met
 4380 4385 4390

ATG CCT GAG TGC CAG TGC CCG CCC CAT ATG ACA GGA CCC CGG TGC CAG 13671
 Met Pro Glu Cys Gln Cys Pro Pro His Met Thr Gly Pro Arg Cys Gln
 4395 4400 4405 4410

GAG CAG GTT GTT AGT CAG CAA CAG CCT GGG CAT ATG GCC TCC ATC CTG 13719
 Glu Gln Val Val Ser Gln Gln Gln Pro Gly His Met Ala Ser Ile Leu
 4415 4420 4425

ATC CCT CTG CTG CTG CTT CTC CTG CTG CTT CTG GTG GCT GGC GTG GTG 13767
 Ile Pro Leu Leu Leu Leu Leu Leu Leu Leu Val Ala Gly Val Val
 4430 4435 4440

TTC TGG TAT AAG CGG CGA GTC CGA GGG GCT AAG GGC TTC CAG CAC CAG 13815
 Phe Trp Tyr Lys Arg Arg Val Arg Gly Ala Lys Gly Phe Gln His Gln
 4445 4450 4455

CGG ATG ACC AAT GGG GCC ATG AAT GTG GAA ATT GGA AAC CCT ACC TAC 13863
 Arg Met Thr Asn Gly Ala Met Asn Val Glu Ile Gly Asn Pro Thr Tyr
 4460 4465 4470

AAG ATG TAT GAA GGT GGA GAG CCC GAT GAT GTC GGG GGC CTA CTG GAT 13911
 Lys Met Tyr Glu Gly Gly Glu Pro Asp Asp Val Gly Gly Leu Leu Asp
 4475 4480 4485 4490

GCT GAT TTT GCC CTT GAC CCT GAC AAG CCT ACC AAC TTC ACC AAC CCA 13959
 Ala Asp Phe Ala Leu Asp Pro Asp Lys Pro Thr Asn Phe Thr Asn Pro
 4495 4500 4505

GTG TAT GCC ACG CTC TAC ATG GGG GGC CAC GGC AGC CGC CAT TCC CTG 14007
 Val Tyr Ala Thr Leu Tyr Met Gly Gly His Gly Ser Arg His Ser Leu
 4510 4515 4520

GCC AGC ACG GAC GAG AAG CGA GAA CTG CTG GGC CGG GGA CCT GAA GAC 14055
 Ala Ser Thr Asp Glu Lys Arg Glu Leu Leu Gly Arg Gly Pro Glu Asp
 4525 4530 4535

FIG.6A-26

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GAG ATA GGA GAT CCC TTG GCA TAGGGCCCTG CCCCACGGA TGTCCCAGA AAGC 14110
CCCTGCCAC ATGAGTCTTT CAATGAACCC CCTCCCAGC CGGCCCTTCT CCGGCCCTGC 14170

Glu Ile Gly Asp Pro Leu Ala

4540

4545

CGGGTGTACA AATGTAAAAA TGAAGGAATT ACTTTTTATA TGTGAGCGAG CAAGCGAGCA 14230

AGCACAGTAT TATCTCTTTG CATTTCCTTC CTGCCTGCTC CTCAGTATCC CCCCATGCT 14290
GCCTTGAGGG GCGGGGAGG GCTTTGTGGC TCAAAGGTAT GAAGGAGTCC ACATGTTCCC 14350
TACCGAGCAT ACCCTGGAA GCCTGGCGG ACGGCCTCCC CACCACGCCT GTGCAAGACA 14410
CTCAACGGGG CTCCGTGTCC CAGCTTTCCT TTCCTTGGCT CTCTGGGGTT AGTTCAGGGG 14470
AGGTGGAGTC CTCTGCTGAC CCTGTCTGGA AGATTTGGCT CTAGCTGAGG AAGGAGTCTT 14530
TtagTTGAGG GAAGTCACCC CAAACCCAG CTCCACTTT CAGGGGCACC TCTCAGATGG 14590
CCATGCTCAG TATCCCTTCC AGACAGGCC TCCCCTCTCT AGCGCCCCCT CTGTGGCTCC 14650
TAGGGCTGAA CACATTCCTT GGTAAGTGT CCCCAGCCT CCCATCCCC TGAGGGCCAG 14710
GAAGAGTCGG GGCACACCAA GGAAGGGCAA GCGGGCAGCC CCATTTTGGG GACGTGAACG 14770
TTTAAATAAT TTTTGCTGAA TTCCTTTACA ACTAAATAAC ACAGATATTG TTATAAATAA 14830
AATTGTAAAA AAAAAAAAAA

FIG.6A-27

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Met	Leu	Thr	Pro	Pro	Leu	Leu	Leu	Leu	Val	Pro	Leu	Leu	Ser	Ala	Leu
1				5					10					15	
Val	Ser	Gly	Ala	Thr	Met	Asp	Ala	Pro	Lys	Thr	Cys	Ser	Pro	Lys	Gln
			20					25					30		
Phe	Ala	Cys	Arg	Asp	Gln	Ile	Thr	Cys	Ile	Ser	Lys	Gly	Trp	Arg	Cys
		35					40					45			
Asp	Gly	Glu	Arg	Asp	Cys	Pro	Asp	Gly	Ser	Asp	Glu	Ala	Pro	Glu	Ile
	50					55					60				
Cys	Pro	Gln	Ser	Lys	Ala	Gln	Arg	Cys	Pro	Pro	Asn	Glu	His	Ser	Cys
65					70					75				80	
Leu	Gly	Thr	Glu	Leu	Cys	Val	Pro	Met	Ser	Arg	Leu	Cys	Asn	Gly	Ile
				85					90					95	
Gln	Asp	Cys	Met	Asp	Gly	Ser	Asp	Glu	Gly	Ala	His	Cys	Arg	Glu	Leu
			100					105					110		
Arg	Ala	Asn	Cys	Ser	Arg	Met	Gly	Cys	Gln	His	His	Cys	Val	Pro	Thr
		115					120					125			
Pro	Ser	Gly	Pro	Thr	Cys	Tyr	Cys	Asn	Ser	Ser	Phe	Gln	Leu	Glu	Ala
	130					135					140				
Asp	Gly	Lys	Thr	Cys	Lys	Asp	Phe	Asp	Glu	Cys	Ser	Val	Tyr	Gly	Thr
145					150					155				160	
Cys	Ser	Gln	Leu	Cys	Thr	Asn	Thr	Asp	Gly	Ser	Phe	Thr	Cys	Gly	Cys
				165					170				175		
Val	Glu	Gly	Tyr	Leu	Leu	Gln	Pro	Asp	Asn	Arg	Ser	Cys	Lys	Ala	Lys
			180					185					190		
Asn	Glu	Pro	Val	Asp	Arg	Pro	Pro	Val	Leu	Leu	Ile	Ala	Asn	Ser	Gln
	195						200					205			
Asn	Ile	Leu	Ala	Thr	Tyr	Leu	Ser	Gly	Ala	Gln	Val	Ser	Thr	Ile	Thr
	210					215					220				
Pro	Thr	Ser	Thr	Arg	Gln	Thr	Thr	Ala	Met	Asp	Phe	Ser	Tyr	Ala	Asn
225					230					235				240	
Glu	Thr	Val	Cys	Trp	Val	His	Val	Gly	Asp	Ser	Ala	Ala	Gln	Thr	Gln
				245					250				255		
Leu	Lys	Cys	Ala	Arg	Met	Pro	Gly	Leu	Lys	Gly	Phe	Val	Asp	Glu	His
			260					265					270		
Thr	Ile	Asn	Ile	Ser	Leu	Ser	Leu	His	His	Val	Glu	Gln	Met	Ala	Ile
	275						280					285			
Asp	Trp	Leu	Thr	Gly	Asn	Phe	Tyr	Phe	Val	Asp	Asp	Ile	Asp	Asp	Arg
	290					295					300				
Ile	Phe	Val	Cys	Asn	Arg	Asn	Gly	Asp	Thr	Cys	Val	Thr	Leu	Leu	Asp
305					310					315					320

FIG.6B-1

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Leu Glu Leu Tyr Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly
 325 330 335
 Lys Val Phe Phe Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys
 340 345 350
 Asp Met Asp Gly Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val
 355 360 365
 Phe Pro His Gly Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp
 370 375 380
 Ala Asp Ala Tyr Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys
 385 390 395 400
 Gly Arg Gln Thr Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly
 405 410 415
 Leu Thr Val Phe Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala
 420 425 430
 Asn Thr Gln Gln Lys Thr Ser Val Ile Arg Val Asn Arg Phe Asn Ser
 435 440 445
 Thr Glu Tyr Gln Val Val Thr Arg Val Asp Lys Gly Gly Ala Leu His
 450 455 460
 Ile Tyr His Gln Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu
 465 470 475 480
 Asn Asp Gln Tyr Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu
 485 490 495
 Ala Asn Ser His Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser
 500 505 510
 Leu Gly Ser Asp Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe
 515 520 525
 Leu Val Tyr Gly Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met
 530 535 540
 Gly Ala Lys Val Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met
 545 550 555 560
 Asn Pro Arg Ala Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe
 565 570 575
 Ala Asp Thr Thr Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr
 580 585 590
 Glu Arg Glu Thr Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val
 595 600 605
 Ala Val Asp Trp Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro
 610 615 620
 Lys Lys Thr Ile Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg
 625 630 635 640
 Lys Thr Leu Ile Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val
 645 650 655

FIG.6B-2

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Asp	Pro	Leu	Asn	Gly	Trp	Met	Tyr	Trp	Thr	Asp	Trp	Glu	Glu	Asp	Pro
			660					665					670		
Lys	Asp	Ser	Arg	Arg	Gly	Arg	Leu	Glu	Arg	Ala	Trp	Met	Asp	Gly	Ser
		675					680					685			
His	Arg	Asp	Ile	Phe	Val	Thr	Ser	Lys	Thr	Val	Leu	Trp	Pro	Asn	Gly
		690				695					700				
Leu	Ser	Leu	Asp	Ile	Pro	Ala	Gly	Arg	Leu	Tyr	Trp	Val	Asp	Ala	Phe
705					710					715					720
Tyr	Asp	Arg	Ile	Glu	Thr	Ile	Leu	Leu	Asn	Gly	Thr	Asp	Arg	Lys	Ile
			725					730						735	
Val	Tyr	Glu	Gly	Pro	Glu	Leu	Asn	His	Ala	Phe	Gly	Leu	Cys	His	His
			740					745					750		
Gly	Asn	Tyr	Leu	Phe	Trp	Thr	Glu	Tyr	Arg	Ser	Gly	Ser	Val	Tyr	Arg
		755					760					765			
Leu	Glu	Arg	Gly	Val	Ala	Gly	Ala	Pro	Pro	Thr	Val	Thr	Leu	Leu	Arg
	770					775					780				
Ser	Glu	Arg	Pro	Pro	Ile	Phe	Glu	Ile	Arg	Met	Tyr	Asp	Ala	His	Glu
785					790					795					800
Gln	Gln	Val	Gly	Thr	Asn	Lys	Cys	Arg	Val	Asn	Asn	Gly	Gly	Cys	Ser
			805					810						815	
Ser	Leu	Cys	Leu	Ala	Thr	Pro	Gly	Ser	Arg	Gln	Cys	Ala	Cys	Ala	Glu
			820					825					830		
Asp	Gln	Val	Leu	Asp	Thr	Asp	Gly	Val	Thr	Cys	Leu	Ala	Asn	Pro	Ser
		835					840					845			
Tyr	Val	Pro	Pro	Pro	Gln	Cys	Gln	Pro	Gly	Gln	Phe	Ala	Cys	Ala	Asn
	850					855					860				
Asn	Arg	Cys	Ile	Gln	Glu	Arg	Trp	Lys	Cys	Asp	Gly	Asp	Asn	Asp	Cys
865				870						875					880
Leu	Asp	Asn	Ser	Asp	Glu	Ala	Pro	Ala	Leu	Cys	His	Gln	His	Thr	Cys
			885						890					895	
Pro	Ser	Asp	Arg	Phe	Lys	Cys	Glu	Asn	Asn	Arg	Cys	Ile	Pro	Asn	Arg
			900					905					910		
Trp	Leu	Cys	Asp	Gly	Asp	Asn	Asp	Cys	Gly	Asn	Ser	Glu	Asp	Glu	Ser
		915					920					925			
Asn	Ala	Thr	Cys	Ser	Ala	Arg	Thr	Cys	Pro	Pro	Asn	Gln	Phe	Ser	Cys
	930					935					940				
Ala	Ser	Gly	Arg	Cys	Ile	Pro	Ile	Ser	Trp	Thr	Cys	Asp	Leu	Asp	Asp
945					950					955					960
Asp	Cys	Gly	Asp	Arg	Ser	Asp	Glu	Ser	Ala	Ser	Cys	Ala	Tyr	Pro	Thr
			965						970					975	
Cys	Phe	Pro	Leu	Thr	Gln	Phe	Thr	Cys	Asn	Asn	Gly	Arg	Cys	Ile	Asn
			980					985					990		

FIG.6B-3

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Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp
 995 1000 1005
 Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn
 1010 1015 1020
 Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp
 025 1030 1035 1040
 Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala
 1045 1050 1055
 Thr Arg Pro Pro Gly Gly Cys His Ser Asp Glu Phe Gln Cys Pro Leu
 1060 1065 1070
 Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp
 1075 1080 1085
 Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val
 1090 1095 1100
 Cys Asp Pro Asn Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile
 105 1110 1115 1120
 Ser Lys Ala Trp Val Cys Asp Gly Asp Ser Asp Cys Glu Asp Asn Ser
 1125 1130 1135
 Asp Glu Glu Asn Cys Glu Ala Leu Ala Cys Arg Pro Pro Ser His Pro
 1140 1145 1150
 Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp
 1155 1160 1165
 Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp
 1170 1175 1180
 Gln Cys Ser Leu Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala
 185 1190 1195 1200
 Pro Gly Glu Gly Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly
 1205 1210 1215
 Ser Asp Asn His Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu
 1220 1225 1230
 Lys Cys Ser Gln Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser
 1235 1240 1245
 Cys Tyr Glu Gly Trp Val Leu Glu Pro Asp Gly Glu Thr Cys Arg Ser
 1250 1255 1260
 Leu Asp Pro Phe Lys Leu Phe Ile Ile Phe Ser Asn Arg His Glu Ile
 265 1270 1275 1280
 Arg Arg Ile Asp Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly
 1285 1290 1295
 Leu Arg Asn Thr Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu
 1300 1305 1310
 Tyr Trp Thr Asp Ala Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu
 1315 1320 1325

FIG.6B-4

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Asp Asn Gly Ala Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu
 1330 1335 1340
 Ala Thr Pro Glu Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr
 345 1350 1355 1360
 Trp Val Glu Ser Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly
 1365 1370 1375
 Thr Leu Arg Thr Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala
 1380 1385 1390
 Ile Ala Leu Asp Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp
 1395 1400 1405
 Ala Ser Leu Pro Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg
 1410 1415 1420
 Arg Thr Ile His Arg Glu Thr Gly Ser Gly Gly Cys Ala Asn Gly Leu
 425 1430 1435 1440
 Thr Val Asp Tyr Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser
 1445 1450 1455
 Asp Ala Ile Tyr Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val
 1460 1465 1470
 Leu Arg Gly His Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr
 1475 1480 1485
 Gly Gly Glu Val Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys
 1490 1495 1500
 Ala Asn Lys Trp Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn
 505 1510 1515 1520
 Thr Gln Pro Phe Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met
 1525 1530 1535
 Ala Pro Asn Pro Cys Glu Ala Asn Gly Gly Arg Gly Pro Cys Ser His
 1540 1545 1550
 Leu Cys Leu Ile Asn Tyr Asn Arg Thr Val Ser Trp Ala Cys Pro His
 1555 1560 1565
 Leu Met Lys Leu His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys
 1570 1575 1580
 Phe Leu Leu Tyr Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp
 585 1590 1595 1600
 Ala Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp
 1605 1610 1615
 Asn Val Thr Val Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp
 1620 1625 1630
 Ser Asp Val Arg Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr
 1635 1640 1645
 Gly Val Glu Thr Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu
 1650 1655 1660

FIG.6B-5

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Ala Val Asp Trp Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr			
665	1670	1675	1680
Asn Lys Lys Gln Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn			
	1685	1690	1695
Ala Val Val Gln Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro			
	1700	1705	1710
Leu Arg Gly Lys Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala			
	1715	1720	1725
Asn Met Asp Gly Ser Asn His Thr Leu Leu Phe Ser Gly Gln Lys Gly			
	1730	1735	1740
Pro Val Gly Leu Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile			
745	1750	1755	1760
Ser Ser Gly Asn His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Glu			
	1765	1770	1775
Leu Glu Val Ile Asp Thr Met Arg Ser Gln Leu Gly Lys Ala Thr Ala			
	1780	1785	1790
Leu Ala Ile Met Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu			
	1795	1800	1805
Lys Met Gly Thr Cys Asn Lys Ala Asp Gly Ser Gly Ser Val Val Leu			
	1810	1815	1820
Arg Asn Ser Thr Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser			
825	1830	1835	1840
Ile Gln Leu Glu His Glu Gly Thr Asn Pro Cys Ser Val Asn Asn Gly			
	1845	1850	1855
Asp Cys Ser Gln Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys			
	1860	1865	1870
Met Cys Thr Ala Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu			
	1875	1880	1885
Gly Val Gly Ser Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly			
	1890	1895	1900
Ile Pro Leu Asp Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser			
905	1910	1915	1920
Gly Thr Ser Leu Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr			
	1925	1930	1935
Ile Tyr Trp Val Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg			
	1940	1945	1950
Asp Gln Thr Trp Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val			
	1955	1960	1965
Glu Gly Ile Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp			
	1970	1975	1980
Gln Gly Phe Asp Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg			
985	1990	1995	2000

FIG.6B-6

SUBSTITUTE SHEET (RULE 26)

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Tyr Val Val Ile Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val
 2005 2010 2015
 His Pro Glu Lys Gly Tyr Leu Phe Trp Thr Glu Trp Gly His Tyr Pro
 2020 2025 2030
 Arg Ile Glu Arg Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val
 2035 2040 2045
 Asn Val Ser Ile Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Gly
 2050 2055 2060
 Gly Lys Leu Tyr Trp Cys Asp Ala Arg Met Asp Lys Ile Glu Arg Ile
 065 2070 2075 2080
 Asp Leu Glu Thr Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn
 2085 2090 2095
 Met Asp Met Phe Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser
 2100 2105 2110
 Asp Arg Thr His Ala Asn Gly Ser Ile Lys Arg Gly Cys Lys Asp Asn
 2115 2120 2125
 Ala Thr Asp Ser Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys
 2130 2135 2140
 Asp Ile Lys Val Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys
 145 2150 2155 2160
 Ala Val Ala Asn Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Gly
 2165 2170 2175
 Gly Gln Arg Ala Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly
 2180 2185 2190
 Ala Ser Cys Arg Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr
 2195 2200 2205
 Ile Leu Lys Ser Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro
 2210 2215 2220
 Val Gln Pro Phe Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu
 225 2230 2235 2240
 Ala Phe Asp Tyr Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile
 2245 2250 2255
 Phe Phe Ser Asp Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp
 2260 2265 2270
 Gly Ser Gly Arg Thr Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly
 2275 2280 2285
 Leu Ala Tyr His Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr
 2290 2295 2300
 Thr Ser Thr Ile Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala
 305 2310 2315 2320
 Phe Glu Arg Glu Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg
 2325 2330 2335

FIG.6B-7

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Ala Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp
 2340 2345 2350
 Asn Glu Leu His Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn
 2355 2360 2365
 Val Leu Thr Leu Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala
 2370 2375 2380
 Ile Asp His Arg Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp
 385 2390 2395 2400
 Lys Ile Glu Arg Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu
 2405 2410 2415
 Lys Ser Glu Pro Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His
 2420 2425 2430
 Ile Phe Trp Thr Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys
 2435 2440 2445
 Tyr Val Gly Ser Asp Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln
 2450 2455 2460
 Pro Met Gly Ile Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu
 465 2470 2475 2480
 Ser Pro Cys Arg Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu
 2485 2490 2495
 Thr His Gln Gly His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu
 2500 2505 2510
 Gln Glu Asp Phe Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln
 2515 2520 2525
 Asp Glu Phe Glu Cys Ala Asn Gly Glu Cys Ile Ser Phe Ser Leu Thr
 2530 2535 2540
 Cys Asp Gly Val Ser His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser
 545 2550 2555 2560
 Tyr Cys Asn Ser Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Asn Asn
 2565 2570 2575
 Gly Arg Cys Val Ser Asn Met Leu Trp Cys Asn Gly Val Asp Tyr Cys
 2580 2585 2590
 Gly Asp Gly Ser Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val
 2595 2600 2605
 Gly Glu Phe Arg Cys Arg Asp Gly Ser Cys Ile Gly Asn Ser Ser Arg
 2610 2615 2620
 Cys Asn Gln Phe Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys
 625 2630 2635 2640
 Ser Ala Thr Asp Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val
 2645 2650 2655
 Leu Phe Gln Pro Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp
 2660 2665 2670

FIG. 6B-8

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Val Cys Asp Gly Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp
 2675 2680 2685
 Cys Pro Gly Val Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys
 2690 2695 2700
 Pro Ser Gly Arg Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp
 705 2710 2715 2720
 Asp Cys Glu Asn Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser
 2725 2730 2735
 Glu Ala Gln Phe Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp
 2740 2745 2750
 Leu Cys Asp Gly Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala
 2755 2760 2765
 His Cys Glu Gly Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly
 2770 2775 2780
 Thr His Val Cys Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp
 785 2790 2795 2800
 Cys Thr Asp Gly Ala Asp Glu Ser Val Thr Ala Gly Cys Leu Tyr Asn
 2805 2810 2815
 Ser Thr Cys Asp Asp Arg Glu Phe Met Cys Gln Asn Arg Leu Cys Ile
 2820 2825 2830
 Pro Lys His Phe Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser
 2835 2840 2845
 Asp Glu Ser Pro Glu Cys Glu Tyr Pro Thr Cys Gly Pro Asn Glu Phe
 2850 2855 2860
 Arg Cys Ala Asn Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp
 865 2870 2875 2880
 Gly Glu Asn Asp Cys His Asp His Ser Asp Glu Ala Pro Lys Asn Pro
 2885 2890 2895
 His Cys Thr Ser Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu
 2900 2905 2910
 Cys Ser Ser Gly Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln
 2915 2920 2925
 Asp Asp Cys Gly Asp Gly Ser Asp Glu Arg Gly Cys His Val Asn Glu
 2930 2935 2940
 Cys Leu Ser Arg Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu
 945 2950 2955 2960
 Lys Ile Gly Phe Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp
 2965 2970 2975
 Asp Gly Arg Thr Cys Ala Asp Leu Asp Glu Cys Ser Thr Thr Phe Pro
 2980 2985 2990
 Cys Ser Gln Leu Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys
 2995 3000 3005

FIG. 6B-9

SUBSTITUTE SHEET (RULE 26)

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Val Glu Gly Tyr Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala
 3010 3015 3020
 Val Thr Asp Glu Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu
 025 3030 3035 3040
 Arg Lys Leu Asn Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly
 3045 3050 3055
 Leu Asn Asn Ala Val Ala Leu Ala Phe Asp Tyr Arg Glu Gln Met Ile
 3060 3065 3070
 Tyr Trp Thr Gly Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His
 3075 3080 3085
 Leu Asn Gly Ser Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn
 3090 3095 3100
 Pro Asp Gly Leu Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys
 105 3110 3115 3120
 Asp Lys Gly Arg Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr
 3125 3130 3135
 Arg Thr Val Leu Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val
 3140 3145 3150
 Val Asp Val Gln Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His
 3155 3160 3165
 Ser Leu Ile Gly Arg Ile Gly Met Asp Gly Ser Gly Arg Ser Ile Ile
 3170 3175 3180
 Val Asp Thr Lys Ile Thr Trp Pro Asn Gly Leu Thr Val Asp Tyr Val
 185 3190 3195 3200
 Thr Glu Arg Ile Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe
 3205 3210 3215
 Ala Ser Leu Asp Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile
 3220 3225 3230
 Pro His Ile Phe Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr
 3235 3240 3245
 Asp Trp Glu Thr Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Ala
 3250 3255 3260
 Asn Lys Thr Leu Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His
 265 3270 3275 3280
 Val Phe His Ala Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys
 3285 3290 3295
 Val Asn Asn Gly Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly
 3300 3305 3310
 Gly His Lys Cys Ala Cys Pro Thr Asn Phe Tyr Leu Gly Gly Asp Gly
 3315 3320 3325
 Arg Thr Cys Val Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn
 3330 3335 3340

FIG.6B-10

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Asp Lys Cys Ile Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys
 345 3350 3355 3360
 Gly Asp His Ser Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg
 3365 3370 3375
 Pro Gly Gln Phe Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe
 3380 3385 3390
 Ile Cys Asp Gly Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn
 3395 3400 3405
 Cys Asp Ile His Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr
 3410 3415 3420
 Asn Arg Cys Ile Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys
 425 3430 3435 3440
 Gly Asp Gly Glu Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro
 3445 3450 3455
 Asn Gln Phe Gln Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp
 3460 3465 3470
 Val Cys Asp Arg Asp Asn His Cys Val Asp Gly Ser Asp Glu Pro Ala
 3475 3480 3485
 Asn Cys Thr Gln Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp
 3490 3495 3500
 Ser Gly Arg Cys Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp
 505 3510 3515 3520
 Cys Gly Asp Gly Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr
 3525 3530 3535
 Cys Glu Pro Tyr Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly
 3540 3545 3550
 Arg Trp Gln Cys Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu
 3555 3560 3565
 Glu Ser Cys Thr Pro Arg Pro Cys Ser Glu Ser Glu Phe Phe Cys Ala
 3570 3575 3580
 Asn Gly Arg Cys Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp
 585 3590 3595 3600
 Cys Ala Asp Gly Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met
 3605 3610 3615
 Asp Gln Phe Gln Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Pro
 3620 3625 3630
 Cys Asp Ala Asp Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys
 3635 3640 3645
 Gly Thr Gly Val Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn
 3650 3655 3660
 Thr Leu Cys Lys Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys
 665 3670 3675 3680

FIG.6B-11

SUBSTITUTE SHEET (RULE 26)

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Gly Asp Asn Ser Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Ile Cys
 3685 3690 3695
 Pro Pro Asn Arg Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp
 3700 3705 3710
 Ile Gly Arg Gln Cys Asp Gly Val Asp Asn Cys Gly Asp Gly Thr Asp
 3715 3720 3725
 Glu Glu Asp Cys Glu Pro Pro Thr Ala Gln Asn Pro His Cys Lys Asp
 3730 3735 3740
 Lys Lys Glu Phe Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu
 745 3750 3755 3760
 Arg Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp
 3765 3770 3775
 Cys Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Met
 3780 3785 3790
 Cys Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys
 3795 3800 3805
 Ala Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln
 3810 3815 3820
 Asp Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Trp Asn
 825 3830 3835 3840
 Lys Pro Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys
 3845 3850 3855
 Thr His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr
 3860 3865 3870
 Ile Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His
 3875 3880 3885
 Ser Ala Tyr Glu Gln Thr Phe Gln Gly Asp Glu Ser Val Arg Ile Asp
 3890 3895 3900
 Ala Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp
 905 3910 3915 3920
 His Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro
 3925 3930 3935
 Thr Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His
 3940 3945 3950
 Leu Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp
 3955 3960 3965
 Val Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu
 3970 3975 3980
 Val Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met
 985 3990 3995 4000
 Ile Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met
 4005 4010 4015

FIG.6B-12

SUBSTITUTE SHEET (RULE 26)

50/65

Tyr Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met
 4020 4025 4030
 Asp Gly Thr Leu Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro
 4035 4040 4045
 Thr Gly Leu Ala Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp
 4050 4055 4060
 Ala Lys Leu Ser Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro
 065 4070 4075 4080
 Ile Val Ala Ala Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile
 4085 4090 4095
 Asp Val Phe Glu Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg
 4100 4105 4110
 Val Phe Lys Ile His Lys Phe Gly His Ser Pro Leu Tyr Asn Leu Thr
 4115 4120 4125
 Gly Gly Leu Ser His Ala Ser Asp Val Val Leu Tyr His Gln His Lys
 4130 4135 4140
 Gln Pro Glu Val Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu
 145 4150 4155 4160
 Cys Leu Leu Ser Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys
 4165 4170 4175
 Arg Leu Asp Asn Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro
 4180 4185 4190
 Pro Asp Ala Pro Arg Pro Gly Thr Cys Thr Leu Gln Cys Phe Asn Gly
 4195 4200 4205
 Gly Ser Cys Phe Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln
 4210 4215 4220
 Pro Arg Tyr Thr Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu Tyr
 225 4230 4235 4240
 Cys His Asn Gly Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr
 4245 4250 4255
 Cys Arg Cys Pro Thr Gly Phe Thr Gly Pro Lys Cys Thr Ala Gln Val
 4260 4265 4270
 Cys Ala Gly Tyr Cys Ser Asn Asn Ser Thr Cys Thr Val Asn Gln Gly
 4275 4280 4285
 Asn Gln Pro Gln Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys
 4290 4295 4300
 Gln Tyr Arg Gln Cys Ser Gly Phe Cys Glu Asn Phe Gly Thr Cys Gln
 305 4310 4315 4320
 Met Ala Ala Asp Gly Ser Arg Gln Cys Arg Cys Thr Val Tyr Phe Glu
 4325 4330 4335
 Gly Pro Arg Cys Glu Val Asn Lys Cys Ser Arg Cys Leu Gln Gly Ala
 4340 4345 4350

FIG.6B-13

SUBSTITUTE SHEET (RULE 26)

51/65

Cys Val Val Asn Lys Gln Thr Gly Asp Val Thr Cys Asn Cys Thr Asp
 4355 4360 4365
 Gly Arg Val Ala Pro Ser Cys Leu Thr Cys Ile Asp His Cys Ser Asn
 4370 4375 4380
 Gly Gly Ser Cys Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys
 385 4390 4395 4400
 Pro Pro His Met Thr Gly Pro Arg Cys Gln Glu Gln Val Val Ser Gln
 4405 4410 4415
 Gln Gln Pro Gly His Met Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu
 4420 4425 4430
 Leu Leu Leu Leu Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg
 4435 4440 4445
 Val Arg Gly Ala Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala
 4450 4455 4460
 Met Asn Val Glu Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly
 465 4470 4475 4480
 Glu Pro Asp Asp Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp
 4485 4490 4495
 Pro Asp Lys Pro Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr
 4500 4505 4510
 Met Gly Gly His Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys
 4515 4520 4525
 Arg Glu Leu Leu Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu
 4530 4535 4540
 Ala
 545

FIG.6B-14

52/65

GCTACAATCC ATCTGGTCTC CTCCAGCTCC TTCTTTCTGC AAC ATG GGG AAG AAC	55
Met Gly Lys Asn	
1	
AAA CTC CTT CAT CCA AGT CTG GTT CTT CTC CTC TTG GTC CTC CTG CCC	103
Lys Leu Leu His Pro Ser Leu Val Leu Leu Leu Leu Val Leu Leu Pro	
5 10 15 20	
ACA GAC GCC TCA GTC TCT GGA AAA CCG CAG TAT ATG GTT CTG GTC CCC	151
Thr Asp Ala Ser Val Ser Gly Lys Pro Gln Tyr Met Val Leu Val Pro	
25 30 35	
TCC CTG CTC CAC ACT GAG ACC ACT GAG AAG GGC TGT GTC CTT CTG AGC	199
Ser Leu Leu His Thr Glu Thr Thr Glu Lys Gly Cys Val Leu Leu Ser	
40 45 50	
TAC CTG AAT GAG ACA GTG ACT GTA AGT GCT TCC TTG GAG TCT GTC AGG	247
Tyr Leu Asn Glu Thr Val Thr Val Ser Ala Ser Leu Glu Ser Val Arg	
55 60 65	
GGA AAC AGG AGC CTC TTC ACT GAC CTG GAG GCG GAG AAT GAC GTA CTC	295
Gly Asn Arg Ser Leu Phe Thr Asp Leu Glu Ala Glu Asn Asp Val Leu	
70 75 80	
CAC TGT GTC GCC TTC GCT GTC CCA AAG TCT TCA TCC AAT GAG GAG GTA	343
His Cys Val Ala Phe Ala Val Pro Lys Ser Ser Ser Asn Glu Glu Val	
85 90 95 100	
ATG TTC CTC ACT GTC CAA GTG AAA GGA CCA ACC CAA GAA TTT AAG AAG	391
Met Phe Leu Thr Val Gln Val Lys Gly Pro Thr Gln Glu Phe Lys Lys	
105 110 115	
CGG ACC ACA GTG ATG GTT AAG AAC GAG GAC AGT CTG GTC TTT GTC CAG	439
Arg Thr Thr Val Met Val Lys Asn Glu Asp Ser Leu Val Phe Val Gln	
120 125 130	
ACA GAC AAA TCA ATC TAC AAA CCA GGG CAG ACA GTG AAA TTT CGT GTT	487
Thr Asp Lys Ser Ile Tyr Lys Pro Gly Gln Thr Val Lys Phe Arg Val	
135 140 145	
GTC TCC ATG GAT GAA AAC TTT CAC CCC CTG AAT GAG TTG ATT CCA CTA	535
Val Ser Met Asp Glu Asn Phe His Pro Leu Asn Glu Leu Ile Pro Leu	
150 155 160	

FIG.7A-1

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GTA TAC ATT CAG GAT CCC AAA GGA AAT CGC ATC GCA CAA TGG CAG AGT Val Tyr Ile Gln Asp Pro Lys Gly Asn Arg Ile Ala Gln Trp Gln Ser 165 170 175 180	583
TTC CAG TTA GAG GGT GGC CTC AAG CAA TTT TCT TTT CCC CTC TCA TCA Phe Gln Leu Glu Gly Gly Leu Lys Gln Phe Ser Phe Pro Leu Ser Ser 185 190 195	631
GAG CCC TTC CAG GGC TCC TAC AAG GTG GTG GTA CAG AAG AAA TCA GGT Glu Pro Phe Gln Gly Ser Tyr Lys Val Val Val Gln Lys Lys Ser Gly 200 205 210	679
GGA AGG ACA GAG CAC CCT TTC ACC GTG GAG GAA TTT GTT CTT CCC AAG Gly Arg Thr Glu His Pro Phe Thr Val Glu Glu Phe Val Leu Pro Lys 215 220 225	727
TTT GAA GTA CAA GTA ACA GTG CCA AAG ATA ATC ACC ATC TTG GAA GAA Phe Glu Val Gln Val Thr Val Pro Lys Ile Ile Thr Ile Leu Glu Glu 230 235 240	775
GAG ATG AAT GTA TCA GTG TGT GGC CTA TAC ACA TAT GGG AAG CCT GTC Glu Met Asn Val Ser Val Cys Gly Leu Tyr Thr Tyr Gly Lys Pro Val 245 250 255 260	823
CCT GGA CAT GTG ACT GTG AGC ATT TGC AGA AAG TAT AGT GAC GCT TCC Pro Gly His Val Thr Val Ser Ile Cys Arg Lys Tyr Ser Asp Ala Ser 265 270 275	871
GAC TGC CAC GGT GAA GAT TCA CAG GCT TTC TGT GAG AAA TTC AGT GGA Asp Cys His Gly Glu Asp Ser Gln Ala Phe Cys Glu Lys Phe Ser Gly 280 285 290	919
CAG CTA AAC AGC CAT GGC TGC TTC TAT CAG CAA GTA AAA ACC AAG GTC Gln Leu Asn Ser His Gly Cys Phe Tyr Gln Gln Val Lys Thr Lys Val 295 300 305	967
TTC CAG CTG AAG AGG AAG GAG TAT GAA ATG AAA CTT CAC ACT GAG GCC Phe Gln Leu Lys Arg Lys Glu Tyr Glu Met Lys Leu His Thr Glu Ala 310 315 320	1015
CAG ATC CAA GAA GAA GGA ACA GTG GTG GAA TTG ACT GGA AGG CAG TCC Gln Ile Gln Glu Glu Gly Thr Val Val Glu Leu Thr Gly Arg Gln Ser 325 330 335 340	1063

FIG.7A-2

54/65

AGT GAA ATC ACA AGA ACC ATA ACC AAA CTC TCA TTT GTG AAA GTG GAC Ser Glu Ile Thr Arg Thr Ile Thr Lys Leu Ser Phe Val Lys Val Asp 345 350 355	1111
TCA CAC TTT CGA CAG GGA ATT CCC TTC TTT GGG CAG GTG CGC CTA GTA Ser His Phe Arg Gln Gly Ile Pro Phe Phe Gly Gln Val Arg Leu Val 360 365 370	1159
GAT GGG AAA GGC GTC CCT ATA CCA AAT AAA GTC ATA TTC ATC AGA GGA Asp Gly Lys Gly Val Pro Ile Pro Asn Lys Val Ile Phe Ile Arg Gly 375 380 385	1207
AAT GAA GCA AAC TAT TAC TCC AAT GCT ACC ACG GAT GAG CAT GGC CTT Asn Glu Ala Asn Tyr Tyr Ser Asn Ala Thr Thr Asp Glu His Gly Leu 390 395 400	1255
GTA CAG TTC TCT ATC AAC ACC ACC AAC GTT ATG GGT ACC TCT CTT ACT Val Gln Phe Ser Ile Asn Thr Thr Asn Val Met Gly Thr Ser Leu Thr 405 410 415 420	1303
GTT AGG GTC AAT TAC AAG GAT CGT AGT CCC TGT TAC GGC TAC CAG TGG Val Arg Val Asn Tyr Lys Asp Arg Ser Pro Cys Tyr Gly Tyr Gln Trp 425 430 435	1351
GTG TCA GAA GAA CAC GAA GAG GCA CAT CAC ACT GCT TAT CTT GTG TTC Val Ser Glu Glu His Glu Glu Ala His His Thr Ala Tyr Leu Val Phe 440 445 450	1399
TCC CCA AGC AAG AGC TTT GTC CAC CTT GAG CCC ATG TCT CAT GAA CTA Ser Pro Ser Lys Ser Phe Val His Leu Glu Pro Met Ser His Glu Leu 455 460 465	1447
CCC TGT GGC CAT ACT CAG ACA GTC CAG GCA CAT TAT ATT CTG AAT GGA Pro Cys Gly His Thr Gln Thr Val Gln Ala His Tyr Ile Leu Asn Gly 470 475 480	1495
GGC ACC CTG CTG GGG CTG AAG AAG CTC TCC TTT TAT TAT CTG ATA ATG Gly Thr Leu Leu Gly Leu Lys Lys Leu Ser Phe Tyr Tyr Leu Ile Met 485 490 495 500	1543
GCA AAG GGA GGC ATT GTC CGA ACT GGG ACT CAT GGA CTG CTT GTG AAG Ala Lys Gly Gly Ile Val Arg Thr Gly Thr His Gly Leu Leu Val Lys 505 510 515	1591

FIG.7A-3

55/65

CAG GAA GAC ATG AAG GGC CAT TTT TCC ATC TCA ATC CCT GTG AAG TCA Gln Glu Asp Met Lys Gly His Phe Ser Ile Ser Ile Pro Val Lys Ser 520 525 530	1639
GAC ATT GCT CCT GTC GCT CGG TTG CTC ATC TAT GCT GTT TTA CCT ACC Asp Ile Ala Pro Val Ala Arg Leu Leu Ile Tyr Ala Val Leu Pro Thr 535 540 545	1687
GGG GAC GTG ATT GGG GAT TCT GCA AAA TAT GAT GTT GAA AAT TGT CTG Gly Asp Val Ile Gly Asp Ser Ala Lys Tyr Asp Val Glu Asn Cys Leu 550 555 560	1735
GCC AAC AAG GTG GAT TTG AGC TTC AGC CCA TCA CAA AGT CTC CCA GCC Ala Asn Lys Val Asp Leu Ser Phe Ser Pro Ser Gln Ser Leu Pro Ala 565 570 575 580	1783
TCA CAC GCC CAC CTG CGA GTC ACA GCG GCT CCT CAG TCC GTC TGC GCC Ser His Ala His Leu Arg Val Thr Ala Ala Pro Gln Ser Val Cys Ala 585 590 595	1831
CTC CGT GCT GTG GAC CAA AGC GTG CTG CTC ATG AAG CCT GAT GCT GAG Leu Arg Ala Val Asp Gln Ser Val Leu Leu Met Lys Pro Asp Ala Glu 600 605 610	1879
CTC TCG GCG TCC TCG GTT TAC AAC CTG CTA CCA GAA AAG GAC CTC ACT Leu Ser Ala Ser Ser Val Tyr Asn Leu Leu Pro Glu Lys Asp Leu Thr 615 620 625	1927
GGC TTC CCT GGG CCT TTG AAT GAC CAG GAC GAT GAA GAC TGC ATC AAT Gly Phe Pro Gly Pro Leu Asn Asp Gln Asp Asp Glu Asp Cys Ile Asn 630 635 640	1975
CGT CAT AAT GTC TAT ATT AAT GGA ATC ACA TAT ACT CCA GTA TCA AGT Arg His Asn Val Tyr Ile Asn Gly Ile Thr Tyr Thr Pro Val Ser Ser 645 650 655 660	2023
ACA AAT GAA AAG GAT ATG TAC AGC TTC CTA GAG GAC ATG GGC TTA AAG Thr Asn Glu Lys Asp Met Tyr Ser Phe Leu Glu Asp Met Gly Leu Lys 665 670 675	2071
GCA TTC ACC AAC TCA AAG ATT CGT AAA CCC AAA ATG TGT CCA CAG CTT Ala Phe Thr Asn Ser Lys Ile Arg Lys Pro Lys Met Cys Pro Gln Leu 680 685 690	2119

FIG.7A-4

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CAA CAG TAT GAA ATG CAT GGA CCT GAA GGT CTA CGT GTA GGT TTT TAT Gln Gln Tyr Glu Met His Gly Pro Glu Gly Leu Arg Val Gly Phe Tyr 695 700 705	2167
GAG TCA GAT GTA ATG GGA AGA GGC CAT GCA CGC CTG GTG CAT GTT GAA Glu Ser Asp Val Met Gly Arg Gly His Ala Arg Leu Val His Val Glu 710 715 720	2215
GAG CCT CAC ACG GAG ACC GTA CGA AAG TAC TTC CCT GAG ACA TGG ATC Glu Pro His Thr Glu Thr Val Arg Lys Tyr Phe Pro Glu Thr Trp Ile 725 730 735 740	2263
TGG GAT TTG GTG GTG GTA AAC TCA GCA GGG GTG GCT GAG GTA GGA GTA Trp Asp Leu Val Val Val Asn Ser Ala Gly Val Ala Glu Val Gly Val 745 750 755	2311
ACA GTC CCT GAC ACC ATC ACC GAG TGG AAG GCA GGG GCC TTC TGC CTG Thr Val Pro Asp Thr Ile Thr Glu Trp Lys Ala Gly Ala Phe Cys Leu 760 765 770	2359
TCT GAA GAT GCT GGA CTT GGT ATC TCT TCC ACT GCC TCT CTC CGA GCC Ser Glu Asp Ala Gly Leu Gly Ile Ser Ser Thr Ala Ser Leu Arg Ala 775 780 785	2407
TTC CAG CCC TTC TTT GTG GAG CTT ACA ATG CCT TAC TCT GTG ATT CGT Phe Gln Pro Phe Phe Val Glu Leu Thr Met Pro Tyr Ser Val Ile Arg 790 795 800	2455
GGA GAG GCC TTC ACA CTC AAG GCC ACG GTC CTA AAC TAC CTT CCC AAA Gly Glu Ala Phe Thr Leu Lys Ala Thr Val Leu Asn Tyr Leu Pro Lys 805 810 815 820	2503
TGC ATC CGG GTC AGT GTG CAG CTG GAA GCC TCT CCC GCC TTC CTT GCT Cys Ile Arg Val Ser Val Gln Leu Glu Ala Ser Pro Ala Phe Leu Ala 825 830 835	2551
GTC CCA GTG GAG AAG GAA CAA GCG CCT CAC TGC ATC TGT GCA AAC GGG Val Pro Val Glu Lys Glu Gln Ala Pro His Cys Ile Cys Ala Asn Gly 840 845 850	2599
CGG CAA ACT GTG TCC TGG GCA GTA ACC CCA AAG TCA TTA GGA AAT GTG Arg Gln Thr Val Ser Trp Ala Val Thr Pro Lys Ser Leu Gly Asn Val 855 860 865	2647

FIG.7A-5

57/65

AAT TTC ACT GTG AGC GCA GAG GCA CTA GAG TCT CAA GAG CTG TGT GGG Asn Phe Thr Val Ser Ala Glu Ala Leu Glu Ser Gln Glu Leu Cys Gly 870 875 880	2695
ACT GAG GTG CCT TCA GTT CCT GAA CAC GGA AGG AAA GAC ACA GTC ATC Thr Glu Val Pro Ser Val Pro Glu His Gly Arg Lys Asp Thr Val Ile 885 890 895 900	2743
AAG CCT CTG TTG GTT GAA CCT GAA GGA CTA GAG AAG GAA ACA ACA TTC Lys Pro Leu Leu Val Glu Pro Glu Gly Leu Glu Lys Glu Thr Thr Phe 905 910 915	2791
AAC TCC CTA CTT TGT CCA TCA GGT GGT GAG GTT TCT GAA GAA TTA TCC Asn Ser Leu Leu Cys Pro Ser Gly Gly Glu Val Ser Glu Glu Leu Ser 920 925 930	2839
CTG AAA CTG CCA CCA AAT GTG GTA GAA GAA TCT GCC CGA GCT TCT GTC Leu Lys Leu Pro Pro Asn Val Val Glu Glu Ser Ala Arg Ala Ser Val 935 940 945	2887
TCA GTT TTG GGA GAC ATA TTA GGC TCT GCC ATG CAA AAC ACA CAA AAT Ser Val Leu Gly Asp Ile Leu Gly Ser Ala Met Gln Asn Thr Gln Asn 950 955 960	2935
CTT CTC CAG ATG CCC TAT GGC TGT GGA GAG CAG AAT ATG GTC CTC TTT Leu Leu Gln Met Pro Tyr Gly Cys Gly Glu Gln Asn Met Val Leu Phe 965 970 975 980	2983
GCT CCT AAC ATC TAT GTA CTG GAT TAT CTA AAT GAA ACA CAG CAG CTT Ala Pro Asn Ile Tyr Val Leu Asp Tyr Leu Asn Glu Thr Gln Gln Leu 985 990 995	3031
ACT CCA GAG GTC AAG TCC AAG GCC ATT GGC TAT CTC AAC ACT GGT TAC Thr Pro Glu Val Lys Ser Lys Ala Ile Gly Tyr Leu Asn Thr Gly Tyr 1000 1005 1010	3079
CAG AGA CAG TTG AAC TAC AAA CAC TAT GAT GGC TCC TAC AGC ACC TTT Gln Arg Gln Leu Asn Tyr Lys His Tyr Asp Gly Ser Tyr Ser Thr Phe 1015 1020 1025	3127
GGG GAG CGA TAT GGC AGG AAC CAG GGC AAC ACC TGG CTC ACA GCC TTT Gly Glu Arg Tyr Gly Arg Asn Gln Gly Asn Thr Trp Leu Thr Ala Phe 1030 1035 1040	3175

FIG.7A-6

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58/65

GTT CTG AAG ACT TTT GCC CAA GCT CGA GCC TAC ATC TTC ATC GAT GAA	3223
Val Leu Lys Thr Phe Ala Gln Ala Arg Ala Tyr Ile Phe Ile Asp Glu	
1045 1050 1055 1060	
GCA CAC ATT ACC CAA GCC CTC ATA TGG CTC TCC CAG AGG CAG AAG GAC	3271
Ala His Ile Thr Gln Ala Leu Ile Trp Leu Ser Gln Arg Gln Lys Asp	
1065 1070 1075	
AAT GGC TGT TTC AGG AGC TCT GGG TCA CTG CTC AAC AAT GCC ATA AAG	3319
Asn Gly Cys Phe Arg Ser Ser Gly Ser Leu Leu Asn Asn Ala Ile Lys	
1080 1085 1090	
GGA GGA GTA GAA GAT GAA GTG ACC CTC TCC GCC TAT ATC ACC ATC GCC	3367
Gly Gly Val Glu Asp Glu Val Thr Leu Ser Ala Tyr Ile Thr Ile Ala	
1095 1100 1105	
CTT CTG GAG ATT CCT CTC ACA GTC ACT CAC CCT GTT GTC CGC AAT GCC	3415
Leu Leu Glu Ile Pro Leu Thr Val Thr His Pro Val Val Arg Asn Ala	
1110 1115 1120	
CTG TTT TGC CTG GAG TCA GCC TGG AAG ACA GCA CAA GAA GGG GAC CAT	3463
Leu Phe Cys Leu Glu Ser Ala Trp Lys Thr Ala Gln Glu Gly Asp His	
1125 1130 1135 1140	
GGC AGC CAT GTA TAT ACC AAA GCA CTG CTG GCC TAT GCT TTT GCC CTG	3511
Gly Ser His Val Tyr Thr Lys Ala Leu Leu Ala Tyr Ala Phe Ala Leu	
1145 1150 1155	
GCA GGT AAC CAG GAC AAG AGG AAG GAA GTA CTC AAG TCA CTT AAT GAG	3559
Ala Gly Asn Gln Asp Lys Arg Lys Glu Val Leu Lys Ser Leu Asn Glu	
1160 1165 1170	
GAA GCT GTG AAG AAA GAC AAC TCT GTC CAT TGG GAG CGC CCT CAG AAA	3607
Glu Ala Val Lys Lys Asp Asn Ser Val His Trp Glu Arg Pro Gln Lys	
1175 1180 1185	
CCC AAG GCA CCA GTG GGG CAT TTT TAC GAA CCC CAG GCT CCC TCT GCT	3655
Pro Lys Ala Pro Val Gly His Phe Tyr Glu Pro Gln Ala Pro Ser Ala	
1190 1195 1200	
GAG GTG GAG ATG ACA TCC TAT GTG CTC CTC GCT TAT CTC ACG GCC CAG	3703
Glu Val Glu Met Thr Ser Tyr Val Leu Leu Ala Tyr Leu Thr Ala Gln	
1205 1210 1215 1220	

FIG. 7A-7

59/65

CCA GCC CCA ACC TCG GAG GAC CTG ACC TCT GCA ACC AAC ATC GTG AAG 3751
Pro Ala Pro Thr Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys
1225 1230 1235

TGG ATC ACG AAG CAG CAG AAT GCC CAG GGC GGT TTC TCC TCC ACC CAG 3799
Trp Ile Thr Lys Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln
1240 1245 1250

GAC ACA GTG GTG GCT CTC CAT GCT CTG TCC AAA TAT GGA GCC GCC ACA 3847
Asp Thr Val Val Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr
1255 1260 1265

TTT ACC AGG ACT GGG AAG GCT GCA CAG GTG ACT ATC CAG TCT TCA GGG 3895
Phe Thr Arg Thr Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly
1270 1275 1280

ACA TTT TCC AGC AAA TTC CAA GTG GAC AAC AAC AAT CGC CTG TTA CTG 3943
Thr Phe Ser Ser Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu
1285 1290 1295 1300

CAG CAG GTC TCA TTG CCA GAG CTG CCT GGG GAA TAC AGC ATG AAA GTG 3991
Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val
1305 1310 1315

ACA GGA GAA GGA TGT GTC TAC CTC CAG ACC TCC TTG AAA TAC AAT ATT 4039
Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile
1320 1325 1330

CTC CCA GAA AAG GAA GAG TTC CCC TTT GCT TTA GGA GTG CAG ACT CTG 4087
Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu
1335 1340 1345

CCT CAA ACT TGT GAT GAA CCC AAA GCC CAC ACC AGC TTC CAA ATC TCC 4135
Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser
1350 1355 1360

CTA AGT GTC AGT TAC ACA GGG AGC CGC TCT GCC TCC AAC ATG GCG ATC 4183
Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile
1365 1370 1375 1380

GTT GAT GTG AAG ATG GTC TCT GGC TTC ATT CCC CTG AAG CCA ACA GTG 4231
Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val
1385 1390 1395

FIG. 7A-8

60/65

AAA ATG CTT GAA AGA TCT AAC CAT GTG AGC CGG ACA GAA GTC AGC AGC	4279
Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser	
1400 1405 1410	
AAC CAT GTC TTG ATT TAC CTT GAT AAG GTG TCA AAT CAG ACA CTG AGC	4327
Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser	
1415 1420 1425	
TTG TTC TTC ACG GTT CTG CAA GAT GTC CCA GTA AGA GAT CTC AAA CCA	4375
Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro	
1430 1435 1440	
GCC ATA GTG AAA GTC TAT GAT TAC TAC GAG ACG GAT GAG TTT GCA ATC	4423
Ala Ile Val Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile	
1445 1450 1455 1460	
GCT GAG TAC AAT GCT CCT TGC AGC AAA GAT CTT GGA AAT GCT TGAAGACCA	4474
Ala Glu Tyr Asn Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala	
1465 1470 1	
CAAGGCTGAA AAGTGCTTTG CTGGAGTCCT GTTCTCTGAG CTCCACAGAA GACACGTGTT	4534
TTTGTATCTT TAAAGACTTG ATGAATAAAC ACTTTTCTG GTC	4577

FIG.7A-9

61/65

Ser	Val	Ser	Gly	Lys	Pro	Gln	Tyr	Met	Val	Leu	Val	Pro	Ser	Leu	Leu
1				5					10					15	
His	Thr	Glu	Thr	Thr	Glu	Lys	Gly	Cys	Val	Leu	Leu	Ser	Tyr	Leu	Asn
			20					25					30		
Glu	Thr	Val	Thr	Val	Ser	Ala	Ser	Leu	Glu	Ser	Val	Arg	Gly	Asn	Arg
		35					40					45			
Ser	Leu	Phe	Thr	Asp	Leu	Glu	Ala	Glu	Asn	Asp	Val	Leu	His	Cys	Val
	50					55					60				
Ala	Phe	Ala	Val	Pro	Lys	Ser	Ser	Ser	Asn	Glu	Glu	Val	Met	Phe	Leu
65					70					75					80
Thr	Val	Gln	Val	Lys	Gly	Pro	Thr	Gln	Glu	Phe	Lys	Lys	Arg	Thr	Thr
				85				90						95	
Val	Met	Val	Lys	Asn	Glu	Asp	Ser	Leu	Val	Phe	Val	Gln	Thr	Asp	Lys
			100					105					110		
Ser	Ile	Tyr	Lys	Pro	Gly	Gln	Thr	Val	Lys	Phe	Arg	Val	Val	Ser	Met
	115					120						125			
Asp	Glu	Asn	Phe	His	Pro	Leu	Asn	Glu	Leu	Ile	Pro	Leu	Val	Tyr	Ile
	130					135					140				
Gln	Asp	Pro	Lys	Gly	Asn	Arg	Ile	Ala	Gln	Trp	Gln	Ser	Phe	Gln	Leu
145					150					155					160
Glu	Gly	Gly	Leu	Lys	Gln	Phe	Ser	Phe	Pro	Leu	Ser	Ser	Glu	Pro	Phe
				165				170					175		
Gln	Gly	Ser	Tyr	Lys	Val	Val	Val	Gln	Lys	Lys	Ser	Gly	Gly	Arg	Thr
			180					185					190		
Glu	His	Pro	Phe	Thr	Val	Glu	Glu	Phe	Val	Leu	Pro	Lys	Phe	Glu	Val
		195					200					205			
Gln	Val	Thr	Val	Pro	Lys	Ile	Ile	Thr	Ile	Leu	Glu	Glu	Glu	Met	Asn
	210					215					220				
Val	Ser	Val	Cys	Gly	Leu	Tyr	Thr	Tyr	Gly	Lys	Pro	Val	Pro	Gly	His
225					230					235					240
Val	Thr	Val	Ser	Ile	Cys	Arg	Lys	Tyr	Ser	Asp	Ala	Ser	Asp	Cys	His
				245				250					255		
Gly	Glu	Asp	Ser	Gln	Ala	Phe	Cys	Glu	Lys	Phe	Ser	Gly	Gln	Leu	Asn
			260					265					270		
Ser	His	Gly	Cys	Phe	Tyr	Gln	Gln	Val	Lys	Thr	Lys	Val	Phe	Gln	Leu
		275					280					285			
Lys	Arg	Lys	Glu	Tyr	Glu	Met	Lys	Leu	His	Thr	Glu	Ala	Gln	Ile	Gln
	290					295					300				
Glu	Glu	Gly	Thr	Val	Val	Glu	Leu	Thr	Gly	Arg	Gln	Ser	Ser	Glu	Ile
305					310					315					320

FIG.7B-1

62/65

Thr Arg Thr Ile Thr Lys Leu Ser Phe Val Lys Val Asp Ser His Phe
 325 330 335
 Arg Gln Gly Ile Pro Phe Phe Gly Gln Val Arg Leu Val Asp Gly Lys
 340 345 350
 Gly Val Pro Ile Pro Asn Lys Val Ile Phe Ile Arg Gly Asn Glu Ala
 355 360 365
 Asn Tyr Tyr Ser Asn Ala Thr Thr Asp Glu His Gly Leu Val Gln Phe
 370 375 380
 Ser Ile Asn Thr Thr Asn Val Met Gly Thr Ser Leu Thr Val Arg Val
 385 390 395 400
 Asn Tyr Lys Asp Arg Ser Pro Cys Tyr Gly Tyr Gln Trp Val Ser Glu
 405 410 415
 Glu His Glu Glu Ala His His Thr Ala Tyr Leu Val Phe Ser Pro Ser
 420 425 430
 Lys Ser Phe Val His Leu Glu Pro Met Ser His Glu Leu Pro Cys Gly
 435 440 445
 His Thr Gln Thr Val Gln Ala His Tyr Ile Leu Asn Gly Gly Thr Leu
 450 455 460
 Leu Gly Leu Lys Lys Leu Ser Phe Tyr Tyr Leu Ile Met Ala Lys Gly
 465 470 475 480
 Gly Ile Val Arg Thr Gly Thr His Gly Leu Leu Val Lys Gln Glu Asp
 485 490 495
 Met Lys Gly His Phe Ser Ile Ser Ile Pro Val Lys Ser Asp Ile Ala
 500 505 510
 Pro Val Ala Arg Leu Leu Ile Tyr Ala Val Leu Pro Thr Gly Asp Val
 515 520 525
 Ile Gly Asp Ser Ala Lys Tyr Asp Val Glu Asn Cys Leu Ala Asn Lys
 530 535 540
 Val Asp Leu Ser Phe Ser Pro Ser Gln Ser Leu Pro Ala Ser His Ala
 545 550 555 560
 His Leu Arg Val Thr Ala Ala Pro Gln Ser Val Cys Ala Leu Arg Ala
 565 570 575
 Val Asp Gln Ser Val Leu Leu Met Lys Pro Asp Ala Glu Leu Ser Ala
 580 585 590
 Ser Ser Val Tyr Asn Leu Leu Pro Glu Lys Asp Leu Thr Gly Phe Pro
 595 600 605
 Gly Pro Leu Asn Asp Gln Asp Asp Glu Asp Cys Ile Asn Arg His Asn
 610 615 620
 Val Tyr Ile Asn Gly Ile Thr Tyr Thr Pro Val Ser Ser Thr Asn Glu
 625 630 635 640
 Lys Asp Met Tyr Ser Phe Leu Glu Asp Met Gly Leu Lys Ala Phe Thr
 645 650 655

FIG. 7B-2

SUBSTITUTE SHEET (RULE 26)

63/65

Asn Ser Lys Ile Arg Lys Pro Lys Met Cys Pro Gln Leu Gln Gln Tyr		
660	665	670
Glu Met His Gly Pro Glu Gly Leu Arg Val Gly Phe Tyr Glu Ser Asp		
675	680	685
Val Met Gly Arg Gly His Ala Arg Leu Val His Val Glu Glu Pro His		
690	695	700
Thr Glu Thr Val Arg Lys Tyr Phe Pro Glu Thr Trp Ile Trp Asp Leu		
705	710	715
Val Val Val Asn Ser Ala Gly Val Ala Glu Val Gly Val Thr Val Pro		
725	730	735
Asp Thr Ile Thr Glu Trp Lys Ala Gly Ala Phe Cys Leu Ser Glu Asp		
740	745	750
Ala Gly Leu Gly Ile Ser Ser Thr Ala Ser Leu Arg Ala Phe Gln Pro		
755	760	765
Phe Phe Val Glu Leu Thr Met Pro Tyr Ser Val Ile Arg Gly Glu Ala		
770	775	780
Phe Thr Leu Lys Ala Thr Val Leu Asn Tyr Leu Pro Lys Cys Ile Arg		
785	790	795
Val Ser Val Gln Leu Glu Ala Ser Pro Ala Phe Leu Ala Val Pro Val		
805	810	815
Glu Lys Glu Gln Ala Pro His Cys Ile Cys Ala Asn Gly Arg Gln Thr		
820	825	830
Val Ser Trp Ala Val Thr Pro Lys Ser Leu Gly Asn Val Asn Phe Thr		
835	840	845
Val Ser Ala Glu Ala Leu Glu Ser Gln Glu Leu Cys Gly Thr Glu Val		
850	855	860
Pro Ser Val Pro Glu His Gly Arg Lys Asp Thr Val Ile Lys Pro Leu		
865	870	875
Leu Val Glu Pro Glu Gly Leu Glu Lys Glu Thr Thr Phe Asn Ser Leu		
885	890	895
Leu Cys Pro Ser Gly Gly Glu Val Ser Glu Glu Leu Ser Leu Lys Leu		
900	905	910
Pro Pro Asn Val Val Glu Glu Ser Ala Arg Ala Ser Val Ser Val Leu		
915	920	925
Gly Asp Ile Leu Gly Ser Ala Met Gln Asn Thr Gln Asn Leu Leu Gln		
930	935	940
Met Pro Tyr Gly Cys Gly Glu Gln Asn Met Val Leu Phe Ala Pro Asn		
945	950	955
Ile Tyr Val Leu Asp Tyr Leu Asn Glu Thr Gln Gln Leu Thr Pro Glu		
965	970	975
Val Lys Ser Lys Ala Ile Gly Tyr Leu Asn Thr Gly Tyr Gln Arg Gln		
980	985	990

FIG.7B-3

SUBSTITUTE SHEET (RULE 26)

64/65

Leu Asn Tyr Lys His Tyr Asp Gly Ser Tyr Ser Thr Phe Gly Glu Arg
 995 1000 1005
 Tyr Gly Arg Asn Gln Gly Asn Thr Trp Leu Thr Ala Phe Val Leu Lys
 1010 1015 1020
 Thr Phe Ala Gln Ala Arg Ala Tyr Ile Phe Ile Asp Glu Ala His Ile
 025 1030 1035 1040
 Thr Gln Ala Leu Ile Trp Leu Ser Gln Arg Gln Lys Asp Asn Gly Cys
 1045 1050 1055
 Phe Arg Ser Ser Gly Ser Leu Leu Asn Asn Ala Ile Lys Gly Gly Val
 1060 1065 1070
 Glu Asp Glu Val Thr Leu Ser Ala Tyr Ile Thr Ile Ala Leu Leu Glu
 1075 1080 1085
 Ile Pro Leu Thr Val Thr His Pro Val Val Arg Asn Ala Leu Phe Cys
 1090 1095 1100
 Leu Glu Ser Ala Trp Lys Thr Ala Gln Glu Gly Asp His Gly Ser His
 105 1110 1115 1120
 Val Tyr Thr Lys Ala Leu Leu Ala Tyr Ala Phe Ala Leu Ala Gly Asn
 1125 1130 1135
 Gln Asp Lys Arg Lys Glu Val Leu Lys Ser Leu Asn Glu Glu Ala Val
 1140 1145 1150
 Lys Lys Asp Asn Ser Val His Trp Glu Arg Pro Gln Lys Pro Lys Ala
 1155 1160 1165
 Pro Val Gly His Phe Tyr Glu Pro Gln Ala Pro Ser Ala Glu Val Glu
 1170 1175 1180
 Met Thr Ser Tyr Val Leu Leu Ala Tyr Leu Thr Ala Gln Pro Ala Pro
 185 1190 1195 1200
 Thr Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys Trp Ile Thr
 1205 1210 1215
 Lys Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val
 1220 1225 1230
 Val Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg
 1235 1240 1245
 Thr Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser
 1250 1255 1260
 Ser Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu Gln Gln Val
 265 1270 1275 1280
 Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu
 1285 1290 1295
 Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu
 1300 1305 1310
 Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr
 1315 1320 1325

FIG. 7B-4

SUBSTITUTE SHEET (RULE 26)

65/65

Cys	Asp	Glu	Pro	Lys	Ala	His	Thr	Ser	Phe	Gln	Ile	Ser	Leu	Ser	Val
1330															
Ser	Tyr	Thr	Gly	Ser	Arg	Ser	Ala	Ser	Asn	Met	Ala	Ile	Val	Asp	Val
345															
Lys	Met	Val	Ser	Gly	Phe	Ile	Pro	Leu	Lys	Pro	Thr	Val	Lys	Met	Leu
Glu	Arg	Ser	Asn	His	Val	Ser	Arg	Thr	Glu	Val	Ser	Ser	Asn	His	Val
Leu	Ile	Tyr	Leu	Asp	Lys	Val	Ser	Asn	Gln	Thr	Leu	Ser	Leu	Phe	Phe
Thr	Val	Leu	Gln	Asp	Val	Pro	Val	Arg	Asp	Leu	Lys	Pro	Ala	Ile	Val
1410															
Lys	Val	Tyr	Asp	Tyr	Tyr	Glu	Thr	Asp	Glu	Phe	Ala	Ile	Ala	Glu	Tyr
425															
Asn	Ala	Pro	Cys	Ser	Lys	Asp	Leu	Gly	Asn	Ala					

FIG.7B-5

SEQUENCE LISTING

<110> Antigenics, Inc.

<120> COMPLEXES OF ALPHA (2) MACROGLOBULIN AND ANTIGENIC
MOLECULES FOR IMMUNOTHERAPY

<130> 8449-178-228

<150> 09/625,139

<151> 2000-07-25

<150> 60/209,266

<151> 2000-06-02

<160> 5

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 14849

<212> DNA

<213> Mus musculus

<400> 1

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caattgtgca	tttttgcagc	cggagtcggc	tccgagatgg	ggctgtgagc	ttcgccctgg	180
gagggggaga	ggagcgagga	gtaaagcagg	ggtgaagggt	tcgaatttgg	gggcaggggg	240
cgaccccgcg	tcagcaggcc	cttcccaggg	ggctcggaac	tgtaccattt	cacctatgcc	300
cctggttcgc	tttgcttaag	gaaggataag	atagaagagt	cggggagagg	aagataaagg	360
gggaccccc	aattgggggg	ggcgaggaca	agaagtaaca	ggaccagagg	gtgggggctg	420
ctgtttgcat	cgccccacac	catgctgacc	cgccgcttgc	tgctgctcgt	gccgctgctt	480
tcagctctgg	tctccggggc	cactatggat	gcccctaaaa	cttgccagccc	taagcagttt	540
gcctgcagag	accaaatac	ctgtatctca	aagggtctgc	ggtgtgacgg	tgaaagagat	600
tgccccgacg	gctctgatga	agccccctgag	atctgtccac	agagtaaagc	ccagagatgc	660
ccgccaatg	agcacagtgt	tctggggact	gagctatgtg	tccccatgtc	tcgtctctgc	720
aacgggatcc	aggactgcat	ggatggctca	gacgagggtg	ctcactgccg	agagctccga	780
gccaaactgtt	ctcgaatggg	ttgtcaacac	cattgtgtac	ctacaccag	tgggccccacg	840
tgctactgta	acagcagctt	ccagctcag	gcagatggca	agacgtgcaa	agattttgac	900
gagtgttccg	tgtatggcac	ctgcagccag	ctttgcacca	acacagatgg	ctccttcaca	960
tgtggctgtg	ttgaaggcta	cctgctgcaa	ccggacaacc	gctcctgcaa	ggccaagaat	1020
gagccagtag	atcgggccgc	agtgtactgt	attgccaaact	ctcagaacat	cctagctacg	1080
tacctgagtg	gggcccgaag	gtctaccatc	acacccacca	gcacccgaca	aaccacgggc	1140
atggacttca	gttatgccaa	tgagaccgta	tgctgggtgc	acgttgggga	cagtgtctgc	1200
cagacacagc	tcaagtgtgc	ccggatgcct	ggcctgaagg	gctttgtgga	tgagcatacc	1260
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ctgtacggcc	tgaccgtgtt	tgagaactat	ctctacgcca	ccaactcgga	caatgccaac	1740
acgcagcaga	agacgagcgt	gatccgagtg	aaccggttca	acagtaactga	gtaccaggtc	1800
gtcaccgctg	tggacaaggg	tggtgccctg	catatctacc	accagcgacg	ccagccccga	1860
gtgcggagtc	acgcctgtga	gaatgaccag	tacgggaagc	cagggtggctg	ctccgacatc	1920

tgccctcctgg	ccaacagtca	caaggcaagg	acctgcaggt	gcaggtctgg	cttcagcctg	1980
ggaagtgatg	ggaagtcttg	taagaaacct	gaacatgagc	tgctcctcgt	gtatggcaag	2040
ggccgaccag	gcatcattag	aggcatggac	atgggggcca	aggtcccaga	tgagcacatg	2100
atccccatcg	agaaccttat	gaatccacgc	gctctggact	tccacgccga	gaccggcttc	2160
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agagagacta	tcttgaagga	tggcatccac	aatgtggagg	gcgtagccgt	ggactggatg	2280
ggagacaatc	tttactggac	tgatgatggc	cccaagaaga	ccattagtgt	ggccaggctg	2340
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attgtagtgg	atccactcaa	tgggtggatg	tactggacag	actgggagga	ggaccccaag	2460
gacagtccgc	gagggcggct	cgagagggct	tggatggacg	gctcacaccg	agatatcttt	2520
gtcacctcca	agacagtgtc	ttggcccaat	gggctaagcc	tggatatccc	agccggacgc	2580
ctctactggg	tggatgcctt	ctatgaccga	attgagacca	tactgtctca	tggcacagac	2640
cggaagattg	tatatgaggg	tcttgaactg	aatcatgcct	tcggcctgtg	tcaccatggc	2700
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20          25          30
Phe Ala Cys Arg Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys
35          40          45

```

Asp	Gly	Glu	Arg	Asp	Cys	Pro	Asp	Gly	Ser	Asp	Glu	Ala	Pro	Glu	Ile
50						55					60				
Cys	Pro	Gln	Ser	Lys	Ala	Gln	Arg	Cys	Pro	Pro	Asn	Glu	His	Ser	Cys
65					70					75					80
Leu	Gly	Thr	Glu	Leu	Cys	Val	Pro	Met	Ser	Arg	Leu	Cys	Asn	Gly	Ile
				85					90					95	
Gln	Asp	Cys	Met	Asp	Gly	Ser	Asp	Glu	Gly	Ala	His	Cys	Arg	Glu	Leu
			100					105					110		
Arg	Ala	Asn	Cys	Ser	Arg	Met	Gly	Cys	Gln	His	His	Cys	Val	Pro	Thr
		115					120					125			
Pro	Ser	Gly	Pro	Thr	Cys	Tyr	Cys	Asn	Ser	Ser	Phe	Gln	Leu	Glu	Ala
		130					135				140				
Asp	Gly	Lys	Thr	Cys	Lys	Asp	Phe	Asp	Glu	Cys	Ser	Val	Tyr	Gly	Thr
145					150					155					160
Cys	Ser	Gln	Leu	Cys	Thr	Asn	Thr	Asp	Gly	Ser	Phe	Thr	Cys	Gly	Cys
				165					170					175	
Val	Glu	Gly	Tyr	Leu	Leu	Gln	Pro	Asp	Asn	Arg	Ser	Cys	Lys	Ala	Lys
			180					185					190		
Asn	Glu	Pro	Val	Asp	Arg	Pro	Pro	Val	Leu	Leu	Ile	Ala	Asn	Ser	Gln
		195					200					205			
Asn	Ile	Leu	Ala	Thr	Tyr	Leu	Ser	Gly	Ala	Gln	Val	Ser	Thr	Ile	Thr
		210					215					220			
Pro	Thr	Ser	Thr	Arg	Gln	Thr	Thr	Ala	Met	Asp	Phe	Ser	Tyr	Ala	Asn
225					230					235					240
Glu	Thr	Val	Cys	Trp	Val	His	Val	Gly	Asp	Ser	Ala	Ala	Gln	Thr	Gln
				245					250					255	
Leu	Lys	Cys	Ala	Arg	Met	Pro	Gly	Leu	Lys	Gly	Phe	Val	Asp	Glu	His
			260					265					270		
Thr	Ile	Asn	Ile	Ser	Leu	Ser	Leu	His	His	Val	Glu	Gln	Met	Ala	Ile
		275					280					285			
Asp	Trp	Leu	Thr	Gly	Asn	Phe	Tyr	Phe	Val	Asp	Asp	Ile	Asp	Asp	Arg
	290				295					300					
Ile	Phe	Val	Cys	Asn	Arg	Asn	Gly	Asp	Thr	Cys	Val	Thr	Leu	Leu	Asp
305					310					315					320
Leu	Glu	Leu	Tyr	Asn	Pro	Lys	Gly	Ile	Ala	Leu	Asp	Pro	Ala	Met	Gly
				325					330					335	
Lys	Val	Phe	Phe	Thr	Asp	Tyr	Gly	Gln	Ile	Pro	Lys	Val	Glu	Arg	Cys
			340					345					350		
Asp	Met	Asp	Gly	Gln	Asn	Arg	Thr	Lys	Leu	Val	Asp	Ser	Lys	Ile	Val
		355					360					365			
Phe	Pro	His	Gly	Ile	Thr	Leu	Asp	Leu	Val	Ser	Arg	Leu	Val	Tyr	Trp
		370				375					380				
Ala	Asp	Ala	Tyr	Leu	Asp	Tyr	Ile	Glu	Val	Val	Asp	Tyr	Glu	Gly	Lys
385					390					395					400
Gly	Arg	Gln	Thr	Ile	Ile	Gln	Gly	Ile	Leu	Ile	Glu	His	Leu	Tyr	Gly
				405					410					415	
Leu	Thr	Val	Phe	Glu	Asn	Tyr	Leu	Tyr	Ala	Thr	Asn	Ser	Asp	Asn	Ala
			420					425					430		
Asn	Thr	Gln	Gln	Lys	Thr	Ser	Val	Ile	Arg	Val	Asn	Arg	Phe	Asn	Ser
		435					440					445			
Thr	Glu	Tyr	Gln	Val	Val	Thr	Arg	Val	Asp	Lys	Gly	Gly	Ala	Leu	His
	450					455					460				
Ile	Tyr	His	Gln	Arg	Arg	Gln	Pro	Arg	Val	Arg	Ser	His	Ala	Cys	Glu
465					470					475					480
Asn	Asp	Gln	Tyr	Gly	Lys	Pro	Gly	Gly	Cys	Ser	Asp	Ile	Cys	Leu	Leu
				485					490					495	
Ala	Asn	Ser	His	Lys	Ala	Arg	Thr	Cys	Arg	Cys	Arg	Ser	Gly	Phe	Ser
			500					505					510		

Leu Gly Ser Asp Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe
 515 520 525
 Leu Val Tyr Gly Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met
 530 535 540
 Gly Ala Lys Val Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met
 545 550 555 560
 Asn Pro Arg Ala Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe
 565 570 575
 Ala Asp Thr Thr Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr
 580 585 590
 Glu Arg Glu Thr Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val
 595 600 605
 Ala Val Asp Trp Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro
 610 615 620
 Lys Lys Thr Ile Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg
 625 630 635 640
 Lys Thr Leu Ile Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val
 645 650 655
 Asp Pro Leu Asn Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro
 660 665 670
 Lys Asp Ser Arg Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser
 675 680 685
 His Arg Asp Ile Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly
 690 695 700
 Leu Ser Leu Asp Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe
 705 710 715 720
 Tyr Asp Arg Ile Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile
 725 730 735
 Val Tyr Glu Gly Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His
 740 745 750
 Gly Asn Tyr Leu Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg
 755 760 765
 Leu Glu Arg Gly Val Ala Gly Ala Pro Pro Thr Val Thr Leu Leu Arg
 770 775 780
 Ser Glu Arg Pro Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala His Glu
 785 790 795 800
 Gln Gln Val Gly Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser
 805 810 815
 Ser Leu Cys Leu Ala Thr Pro Gly Ser Arg Gln Cys Ala Cys Ala Glu
 820 825 830
 Asp Gln Val Leu Asp Thr Asp Gly Val Thr Cys Leu Ala Asn Pro Ser
 835 840 845
 Tyr Val Pro Pro Pro Gln Cys Gln Pro Gly Gln Phe Ala Cys Ala Asn
 850 855 860
 Asn Arg Cys Ile Gln Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys
 865 870 875 880
 Leu Asp Asn Ser Asp Glu Ala Pro Ala Leu Cys His Gln His Thr Cys
 885 890 895
 Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg
 900 905 910
 Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser
 915 920 925
 Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys
 930 935 940
 Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp
 945 950 955 960
 Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr
 965 970 975

Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn
 980 985 990
 Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp
 995 1000 1005
 Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn
 1010 1015 1020
 Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp
 1025 1030 1035 1040
 Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala
 1045 1050 1055
 Thr Arg Pro Pro Gly Gly Cys His Ser Asp Glu Phe Gln Cys Pro Leu
 1060 1065 1070
 Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp
 1075 1080 1085
 Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val
 1090 1095 1100
 Cys Asp Pro Asn Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile
 1105 1110 1115 1120
 Ser Lys Ala Trp Val Cys Asp Gly Asp Ser Asp Cys Glu Asp Asn Ser
 1125 1130 1135
 Asp Glu Glu Asn Cys Glu Ala Leu Ala Cys Arg Pro Pro Ser His Pro
 1140 1145 1150
 Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp
 1155 1160 1165
 Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp
 1170 1175 1180
 Gln Cys Ser Leu Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala
 1185 1190 1195 1200
 Pro Gly Glu Gly Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly
 1205 1210 1215
 Ser Asp Asn His Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu
 1220 1225 1230
 Lys Cys Ser Gln Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser
 1235 1240 1245
 Cys Tyr Glu Gly Trp Val Leu Glu Pro Asp Gly Glu Thr Cys Arg Ser
 1250 1255 1260
 Leu Asp Pro Phe Lys Leu Phe Ile Ile Phe Ser Asn Arg His Glu Ile
 1265 1270 1275 1280
 Arg Arg Ile Asp Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly
 1285 1290 1295
 Leu Arg Asn Thr Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu
 1300 1305 1310
 Tyr Trp Thr Asp Ala Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu
 1315 1320 1325
 Asp Asn Gly Ala Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu
 1330 1335 1340
 Ala Thr Pro Glu Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr
 1345 1350 1355 1360
 Trp Val Glu Ser Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly
 1365 1370 1375
 Thr Leu Arg Thr Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala
 1380 1385 1390
 Ile Ala Leu Asp Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp
 1395 1400 1405
 Ala Ser Leu Pro Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg
 1410 1415 1420
 Arg Thr Ile His Arg Glu Thr Gly Ser Gly Gly Cys Ala Asn Gly Leu
 1425 1430 1435 1440

Thr Val Asp Tyr Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser
 1445 1450 1455
 Asp Ala Ile Tyr Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val
 1460 1465 1470
 Leu Arg Gly His Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr
 1475 1480 1485
 Gly Gly Glu Val Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys
 1490 1495 1500
 Ala Asn Lys Trp Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn
 1505 1510 1515 1520
 Thr Gln Pro Phe Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met
 1525 1530 1535
 Ala Pro Asn Pro Cys Glu Ala Asn Gly Gly Arg Gly Pro Cys Ser His
 1540 1545 1550
 Leu Cys Leu Ile Asn Tyr Asn Arg Thr Val Ser Trp Ala Cys Pro His
 1555 1560 1565
 Leu Met Lys Leu His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys
 1570 1575 1580
 Phe Leu Leu Tyr Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp
 1585 1590 1595 1600
 Ala Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp
 1605 1610 1615
 Asn Val Thr Val Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp
 1620 1625 1630
 Ser Asp Val Arg Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr
 1635 1640 1645
 Gly Val Glu Thr Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu
 1650 1655 1660
 Ala Val Asp Trp Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr
 1665 1670 1675 1680
 Asn Lys Lys Gln Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn
 1685 1690 1695
 Ala Val Val Gln Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro
 1700 1705 1710
 Leu Arg Gly Lys Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala
 1715 1720 1725
 Asn Met Asp Gly Ser Asn His Thr Leu Leu Phe Ser Gly Gln Lys Gly
 1730 1735 1740
 Pro Val Gly Leu Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile
 1745 1750 1755 1760
 Ser Ser Gly Asn His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Glu
 1765 1770 1775
 Leu Glu Val Ile Asp Thr Met Arg Ser Gln Leu Gly Lys Ala Thr Ala
 1780 1785 1790
 Leu Ala Ile Met Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu
 1795 1800 1805
 Lys Met Gly Thr Cys Asn Lys Ala Asp Gly Ser Gly Ser Val Val Leu
 1810 1815 1820
 Arg Asn Ser Thr Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser
 1825 1830 1835 1840
 Ile Gln Leu Glu His Glu Gly Thr Asn Pro Cys Ser Val Asn Asn Gly
 1845 1850 1855
 Asp Cys Ser Gln Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys
 1860 1865 1870
 Met Cys Thr Ala Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu
 1875 1880 1885
 Gly Val Gly Ser Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly
 1890 1895 1900



Ile Pro Leu Asp Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser
 1905 1910 1915 1920
 Gly Thr Ser Leu Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr
 1925 1930 1935
 Ile Tyr Trp Val Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg
 1940 1945 1950
 Asp Gln Thr Trp Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val
 1955 1960 1965
 Glu Gly Ile Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp
 1970 1975 1980
 Gln Gly Phe Asp Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg
 1985 1990 1995 2000
 Tyr Val Val Ile Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val
 2005 2010 2015
 His Pro Glu Lys Gly Tyr Leu Phe Trp Thr Glu Trp Gly His Tyr Pro
 2020 2025 2030
 Arg Ile Glu Arg Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val
 2035 2040 2045
 Asn Val Ser Ile Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Gly
 2050 2055 2060
 Gly Lys Leu Tyr Trp Cys Asp Ala Arg Met Asp Lys Ile Glu Arg Ile
 2065 2070 2075 2080
 Asp Leu Glu Thr Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn
 2085 2090 2095
 Met Asp Met Phe Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser
 2100 2105 2110
 Asp Arg Thr His Ala Asn Gly Ser Ile Lys Arg Gly Cys Lys Asp Asn
 2115 2120 2125
 Ala Thr Asp Ser Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys
 2130 2135 2140
 Asp Ile Lys Val Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys
 2145 2150 2155 2160
 Ala Val Ala Asn Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Gly
 2165 2170 2175
 Gly Gln Arg Ala Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly
 2180 2185 2190
 Ala Ser Cys Arg Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr
 2195 2200 2205
 Ile Leu Lys Ser Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro
 2210 2215 2220
 Val Gln Pro Phe Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu
 2225 2230 2235 2240
 Ala Phe Asp Tyr Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile
 2245 2250 2255
 Phe Phe Ser Asp Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp
 2260 2265 2270
 Gly Ser Gly Arg Thr Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly
 2275 2280 2285
 Leu Ala Tyr His Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr
 2290 2295 2300
 Thr Ser Thr Ile Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala
 2305 2310 2315 2320
 Phe Glu Arg Glu Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg
 2325 2330 2335
 Ala Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp
 2340 2345 2350
 Asn Glu Leu His Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn
 2355 2360 2365

Val Leu Thr Leu Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala
 2370 2375 2380
 Ile Asp His Arg Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp
 2385 2390 2395 2400
 Lys Ile Glu Arg Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu
 2405 2410 2415
 Lys Ser Glu Pro Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His
 2420 2425 2430
 Ile Phe Trp Thr Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys
 2435 2440 2445
 Tyr Val Gly Ser Asp Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln
 2450 2455 2460
 Pro Met Gly Ile Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu
 2465 2470 2475 2480
 Ser Pro Cys Arg Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu
 2485 2490 2495
 Thr His Gln Gly His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu
 2500 2505 2510
 Gln Glu Asp Phe Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln
 2515 2520 2525
 Asp Glu Phe Glu Cys Ala Asn Gly Glu Cys Ile Ser Phe Ser Leu Thr
 2530 2535 2540
 Cys Asp Gly Val Ser His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser
 2545 2550 2555 2560
 Tyr Cys Asn Ser Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Asn Asn
 2565 2570 2575
 Gly Arg Cys Val Ser Asn Met Leu Trp Cys Asn Gly Val Asp Tyr Cys
 2580 2585 2590
 Gly Asp Gly Ser Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val
 2595 2600 2605
 Gly Glu Phe Arg Cys Arg Asp Gly Ser Cys Ile Gly Asn Ser Ser Arg
 2610 2615 2620
 Cys Asn Gln Phe Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys
 2625 2630 2635 2640
 Ser Ala Thr Asp Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val
 2645 2650 2655
 Leu Phe Gln Pro Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp
 2660 2665 2670
 Val Cys Asp Gly Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp
 2675 2680 2685
 Cys Pro Gly Val Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys
 2690 2695 2700
 Pro Ser Gly Arg Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp
 2705 2710 2715 2720
 Asp Cys Glu Asn Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser
 2725 2730 2735
 Glu Ala Gln Phe Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp
 2740 2745 2750
 Leu Cys Asp Gly Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala
 2755 2760 2765
 His Cys Glu Gly Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly
 2770 2775 2780
 Thr His Val Cys Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp
 2785 2790 2795 2800
 Cys Thr Asp Gly Ala Asp Glu Ser Val Thr Ala Gly Cys Leu Tyr Asn
 2805 2810 2815
 Ser Thr Cys Asp Asp Arg Glu Phe Met Cys Gln Asn Arg Leu Cys Ile
 2820 2825 2830

Pro Lys His Phe Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser
 2835 2840 2845
 Asp Glu Ser Pro Glu Cys Glu Tyr Pro Thr Cys Gly Pro Asn Glu Phe
 2850 2855 2860
 Arg Cys Ala Asn Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp
 2865 2870 2875 2880
 Gly Glu Asn Asp Cys His Asp His Ser Asp Glu Ala Pro Lys Asn Pro
 2885 2890 2895
 His Cys Thr Ser Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu
 2900 2905 2910
 Cys Ser Ser Gly Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln
 2915 2920 2925
 Asp Asp Cys Gly Asp Gly Ser Asp Glu Arg Gly Cys His Val Asn Glu
 2930 2935 2940
 Cys Leu Ser Arg Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu
 2945 2950 2955 2960
 Lys Ile Gly Phe Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp
 2965 2970 2975
 Asp Gly Arg Thr Cys Ala Asp Leu Asp Glu Cys Ser Thr Thr Phe Pro
 2980 2985 2990
 Cys Ser Gln Leu Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys
 2995 3000 3005
 Val Glu Gly Tyr Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala
 3010 3015 3020
 Val Thr Asp Glu Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu
 3025 3030 3035 3040
 Arg Lys Leu Asn Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly
 3045 3050 3055
 Leu Asn Asn Ala Val Ala Leu Ala Phe Asp Tyr Arg Glu Gln Met Ile
 3060 3065 3070
 Tyr Trp Thr Gly Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His
 3075 3080 3085
 Leu Asn Gly Ser Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn
 3090 3095 3100
 Pro Asp Gly Leu Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys
 3105 3110 3115 3120
 Asp Lys Gly Arg Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr
 3125 3130 3135
 Arg Thr Val Leu Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val
 3140 3145 3150
 Val Asp Val Gln Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His
 3155 3160 3165
 Ser Leu Ile Gly Arg Ile Gly Met Asp Gly Ser Gly Arg Ser Ile Ile
 3170 3175 3180
 Val Asp Thr Lys Ile Thr Trp Pro Asn Gly Leu Thr Val Asp Tyr Val
 3185 3190 3195 3200
 Thr Glu Arg Ile Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe
 3205 3210 3215
 Ala Ser Leu Asp Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile
 3220 3225 3230
 Pro His Ile Phe Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr
 3235 3240 3245
 Asp Trp Glu Thr Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Ala
 3250 3255 3260
 Asn Lys Thr Leu Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His
 3265 3270 3275 3280
 Val Phe His Ala Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys
 3285 3290 3295

Val Asn Asn Gly Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly
 3300 3305 3310
 Gly His Lys Cys Ala Cys Pro Thr Asn Phe Tyr Leu Gly Gly Asp Gly
 3315 3320 3325
 Arg Thr Cys Val Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn
 3330 3335 3340
 Asp Lys Cys Ile Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys
 3345 3350 3355 3360
 Gly Asp His Ser Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg
 3365 3370 3375
 Pro Gly Gln Phe Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe
 3380 3385 3390
 Ile Cys Asp Gly Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn
 3395 3400 3405
 Cys Asp Ile His Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr
 3410 3415 3420
 Asn Arg Cys Ile Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys
 3425 3430 3435 3440
 Gly Asp Gly Glu Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro
 3445 3450 3455
 Asn Gln Phe Gln Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp
 3460 3465 3470
 Val Cys Asp Arg Asp Asn His Cys Val Asp Gly Ser Asp Glu Pro Ala
 3475 3480 3485
 Asn Cys Thr Gln Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp
 3490 3495 3500
 Ser Gly Arg Cys Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp
 3505 3510 3515 3520
 Cys Gly Asp Gly Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr
 3525 3530 3535
 Cys Glu Pro Tyr Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly
 3540 3545 3550
 Arg Trp Gln Cys Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu
 3555 3560 3565
 Glu Ser Cys Thr Pro Arg Pro Cys Ser Glu Ser Glu Phe Phe Cys Ala
 3570 3575 3580
 Asn Gly Arg Cys Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp
 3585 3590 3595 3600
 Cys Ala Asp Gly Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met
 3605 3610 3615
 Asp Gln Phe Gln Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Pro
 3620 3625 3630
 Cys Asp Ala Asp Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys
 3635 3640 3645
 Gly Thr Gly Val Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn
 3650 3655 3660
 Thr Leu Cys Lys Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys
 3665 3670 3675 3680
 Gly Asp Asn Ser Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Ile Cys
 3685 3690 3695
 Pro Pro Asn Arg Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp
 3700 3705 3710
 Ile Gly Arg Gln Cys Asp Gly Val Asp Asn Cys Gly Asp Gly Thr Asp
 3715 3720 3725
 Glu Glu Asp Cys Glu Pro Pro Thr Ala Gln Asn Pro His Cys Lys Asp
 3730 3735 3740
 Lys Lys Glu Phe Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu
 3745 3750 3755 3760

Arg Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp
 3765 3770 3775
 Cys Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Met
 3780 3785 3790
 Cys Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys
 3795 3800 3805
 Ala Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln
 3810 3815 3820
 Asp Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Trp Asn
 3825 3830 3835 3840
 Lys Pro Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys
 3845 3850 3855
 Thr His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr
 3860 3865 3870
 Ile Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His
 3875 3880 3885
 Ser Ala Tyr Glu Gln Thr Phe Gln Gly Asp Glu Ser Val Arg Ile Asp
 3890 3895 3900
 Ala Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp
 3905 3910 3915 3920
 His Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro
 3925 3930 3935
 Thr Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His
 3940 3945 3950
 Leu Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp
 3955 3960 3965
 Val Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu
 3970 3975 3980
 Val Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met
 3985 3990 3995 4000
 Ile Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met
 4005 4010 4015
 Tyr Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met
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 Ser His Glu Leu Pro Cys Gly His Thr Gln Thr Val Gln Ala His Tyr
 465 470 475 480
 Ile Leu Asn Gly Gly Thr Leu Leu Gly Leu Lys Lys Leu Ser Phe Tyr
 485 490 495
 Tyr Leu Ile Met Ala Lys Gly Gly Ile Val Arg Thr Gly Thr His Gly
 500 505 510
 Leu Leu Val Lys Gln Glu Asp Met Lys Gly His Phe Ser Ile Ser Ile
 515 520 525
 Pro Val Lys Ser Asp Ile Ala Pro Val Ala Arg Leu Leu Ile Tyr Ala
 530 535 540
 Val Leu Pro Thr Gly Asp Val Ile Gly Asp Ser Ala Lys Tyr Asp Val
 545 550 555 560
 Glu Asn Cys Leu Ala Asn Lys Val Asp Leu Ser Phe Ser Pro Ser Gln
 565 570 575
 Ser Leu Pro Ala Ser His Ala His Leu Arg Val Thr Ala Ala Pro Gln
 580 585 590
 Ser Val Cys Ala Leu Arg Ala Val Asp Gln Ser Val Leu Leu Met Lys
 595 600 605

Pro Asp Ala Glu Leu Ser Ala Ser Ser Val Tyr Asn Leu Leu Pro Glu
 610 615 620
 Lys Asp Leu Thr Gly Phe Pro Gly Pro Leu Asn Asp Gln Asp Asp Glu
 625 630 635 640
 Asp Cys Ile Asn Arg His Asn Val Tyr Ile Asn Gly Ile Thr Tyr Thr
 645 650 655
 Pro Val Ser Ser Thr Asn Glu Lys Asp Met Tyr Ser Phe Leu Glu Asp
 660 665 670
 Met Gly Leu Lys Ala Phe Thr Asn Ser Lys Ile Arg Lys Pro Lys Met
 675 680 685
 Cys Pro Gln Leu Gln Gln Tyr Glu Met His Gly Pro Glu Gly Leu Arg
 690 695 700
 Val Gly Phe Tyr Glu Ser Asp Val Met Gly Arg Gly His Ala Arg Leu
 705 710 715 720
 Val His Val Glu Glu Pro His Thr Glu Thr Val Arg Lys Tyr Phe Pro
 725 730 735
 Glu Thr Trp Ile Trp Asp Leu Val Val Val Asn Ser Ala Gly Val Ala
 740 745 750
 Glu Val Gly Val Thr Val Pro Asp Thr Ile Thr Glu Trp Lys Ala Gly
 755 760 765
 Ala Phe Cys Leu Ser Glu Asp Ala Gly Leu Gly Ile Ser Ser Thr Ala
 770 775 780
 Ser Leu Arg Ala Phe Gln Pro Phe Phe Val Glu Leu Thr Met Pro Tyr
 785 790 795 800
 Ser Val Ile Arg Gly Glu Ala Phe Thr Leu Lys Ala Thr Val Leu Asn
 805 810 815
 Tyr Leu Pro Lys Cys Ile Arg Val Ser Val Gln Leu Glu Ala Ser Pro
 820 825 830
 Ala Phe Leu Ala Val Pro Val Glu Lys Glu Gln Ala Pro His Cys Ile
 835 840 845
 Cys Ala Asn Gly Arg Gln Thr Val Ser Trp Ala Val Thr Pro Lys Ser
 850 855 860
 Leu Gly Asn Val Asn Phe Thr Val Ser Ala Glu Ala Leu Glu Ser Gln
 865 870 875 880
 Glu Leu Cys Gly Thr Glu Val Pro Ser Val Pro Glu His Gly Arg Lys
 885 890 895
 Asp Thr Val Ile Lys Pro Leu Leu Val Glu Pro Glu Gly Leu Glu Lys
 900 905 910
 Glu Thr Thr Phe Asn Ser Leu Leu Cys Pro Ser Gly Gly Glu Val Ser
 915 920 925
 Glu Glu Leu Ser Leu Lys Leu Pro Pro Asn Val Val Glu Glu Ser Ala
 930 935 940
 Arg Ala Ser Val Ser Val Leu Gly Asp Ile Leu Gly Ser Ala Met Gln
 945 950 955 960
 Asn Thr Gln Asn Leu Leu Gln Met Pro Tyr Gly Cys Gly Glu Gln Asn
 965 970 975
 Met Val Leu Phe Ala Pro Asn Ile Tyr Val Leu Asp Tyr Leu Asn Glu
 980 985 990
 Thr Gln Gln Leu Thr Pro Glu Val Lys Ser Lys Ala Ile Gly Tyr Leu
 995 1000 1005
 Asn Thr Gly Tyr Gln Arg Gln Leu Asn Tyr Lys His Tyr Asp Gly Ser
 1010 1015 1020
 Tyr Ser Thr Phe Gly Glu Arg Tyr Gly Arg Asn Gln Gly Asn Thr Trp
 1025 1030 1035 1040
 Leu Thr Ala Phe Val Leu Lys Thr Phe Ala Gln Ala Arg Ala Tyr Ile
 1045 1050 1055
 Phe Ile Asp Glu Ala His Ile Thr Gln Ala Leu Ile Trp Leu Ser Gln
 1060 1065 1070

Arg Gln Lys Asp Asn Gly Cys Phe Arg Ser Ser Gly Ser Leu Leu Asn
 1075 1080 1085
 Asn Ala Ile Lys Gly Gly Val Glu Asp Glu Val Thr Leu Ser Ala Tyr
 1090 1095 1100
 Ile Thr Ile Ala Leu Leu Glu Ile Pro Leu Thr Val Thr His Pro Val
 1105 1110 1115 1120
 Val Arg Asn Ala Leu Phe Cys Leu Glu Ser Ala Trp Lys Thr Ala Gln
 1125 1130 1135
 Glu Gly Asp His Gly Ser His Val Tyr Thr Lys Ala Leu Leu Ala Tyr
 1140 1145 1150
 Ala Phe Ala Leu Ala Gly Asn Gln Asp Lys Arg Lys Glu Val Leu Lys
 1155 1160 1165
 Ser Leu Asn Glu Glu Ala Val Lys Lys Asp Asn Ser Val His Trp Glu
 1170 1175 1180
 Arg Pro Gln Lys Pro Lys Ala Pro Val Gly His Phe Tyr Glu Pro Gln
 1185 1190 1195 1200
 Ala Pro Ser Ala Glu Val Glu Met Thr Ser Tyr Val Leu Leu Ala Tyr
 1205 1210 1215
 Leu Thr Ala Gln Pro Ala Pro Thr Ser Glu Asp Leu Thr Ser Ala Thr
 1220 1225 1230
 Asn Ile Val Lys Trp Ile Thr Lys Gln Gln Asn Ala Gln Gly Gly Phe
 1235 1240 1245
 Ser Ser Thr Gln Asp Thr Val Val Ala Leu His Ala Leu Ser Lys Tyr
 1250 1255 1260
 Gly Ala Ala Thr Phe Thr Arg Thr Gly Lys Ala Ala Gln Val Thr Ile
 1265 1270 1275 1280
 Gln Ser Ser Gly Thr Phe Ser Ser Lys Phe Gln Val Asp Asn Asn Asn
 1285 1290 1295
 Arg Leu Leu Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr
 1300 1305 1310
 Ser Met Lys Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu
 1315 1320 1325
 Lys Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly
 1330 1335 1340
 Val Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser
 1345 1350 1355 1360
 Phe Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser
 1365 1370 1375
 Asn Met Ala Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu
 1380 1385 1390
 Lys Pro Thr Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr
 1395 1400 1405
 Glu Val Ser Ser Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn
 1410 1415 1420
 Gln Thr Leu Ser Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg
 1425 1430 1435 1440
 Asp Leu Lys Pro Ala Ile Val Lys Val Tyr Asp Tyr Tyr Glu Thr Asp
 1445 1450 1455
 Glu Phe Ala Ile Ala Glu Tyr Asn Ala Pro Cys Ser Lys Asp Leu Gly
 1460 1465 1470
 Asn Ala

INTERNATIONAL SEARCH REPORT

Int'l application No.
PCT/US01/18047

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/00, 39/385, 39/39, 47/00, 55/14; C07K 1/02, 1/04
US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 185.1, 193.1, 195.11, 196.11, 197.11; 580/392, 402, 403

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, Embase, Scisearch, WPIDS, USPatfull
search terms: alpha2- macroglobulin, noncovalent complex, molecular complex and alpha globulin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SMORDIN et al. The complex of alpha-2 macroglobulin with CD2 in the plasma of Gastric Carcinoma patients. Scand J Immunol. 1991. Vol. 33. No. 6. pages 699-706. see Abstract.	7-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
12 AUGUST 2001

Date of mailing of the international search report
25 OCT 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20251
Facsimile No. (703) 305-3230

Authorized officer

GEETHA P. BANSAL

Telephone No. (703) 308-0198

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/18047

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/184.1, 185.1, 193.1, 195.11, 196.11, 197.11; 530/392, 402, 403